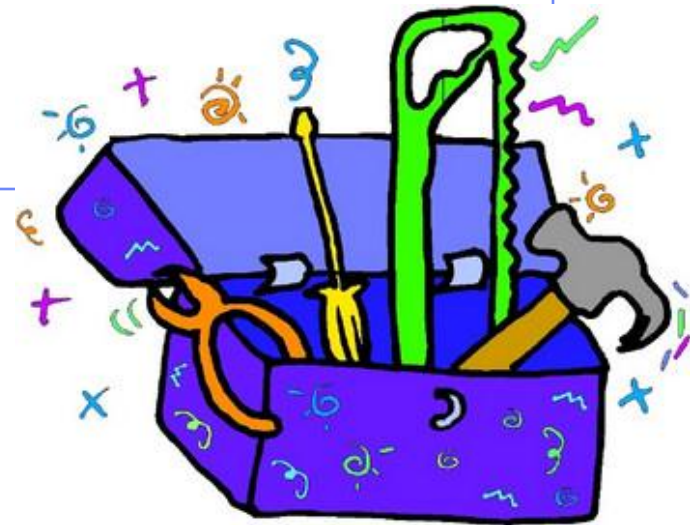
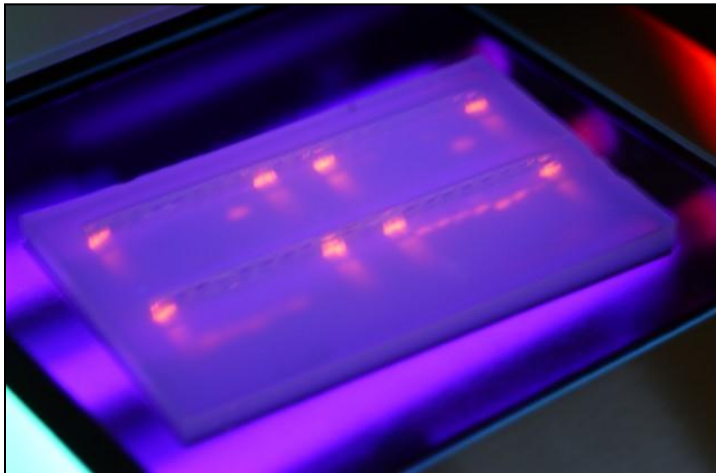


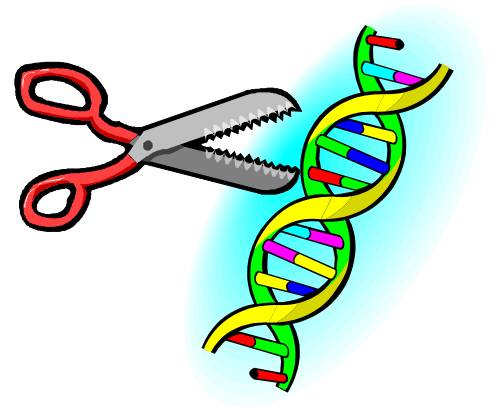
More Basic Biotechnology Tools

Sorting & Copying DNA



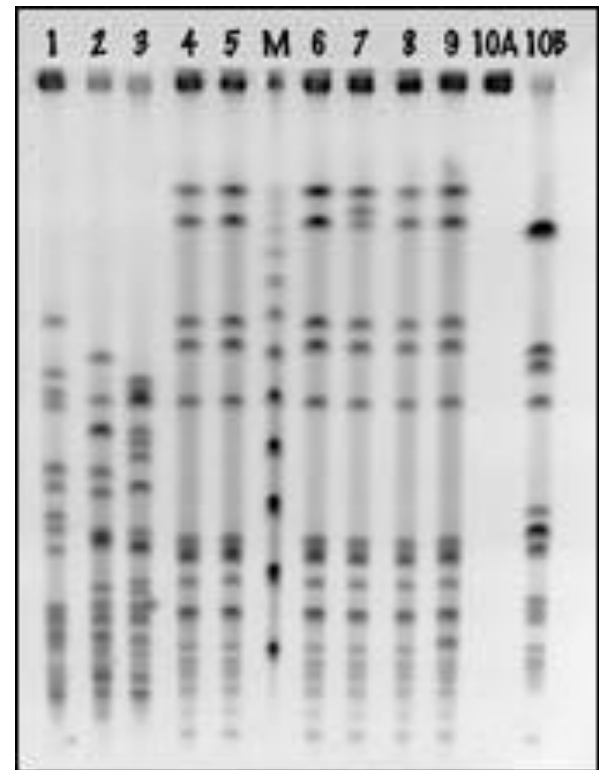
Many uses of restriction enzymes...

- Now that we can cut DNA with restriction enzymes...
 - ◆ we can cut up DNA from different people... or different organisms... and compare it
 - ◆ why?
 - forensics
 - medical diagnostics
 - paternity
 - evolutionary relationships
 - and more...



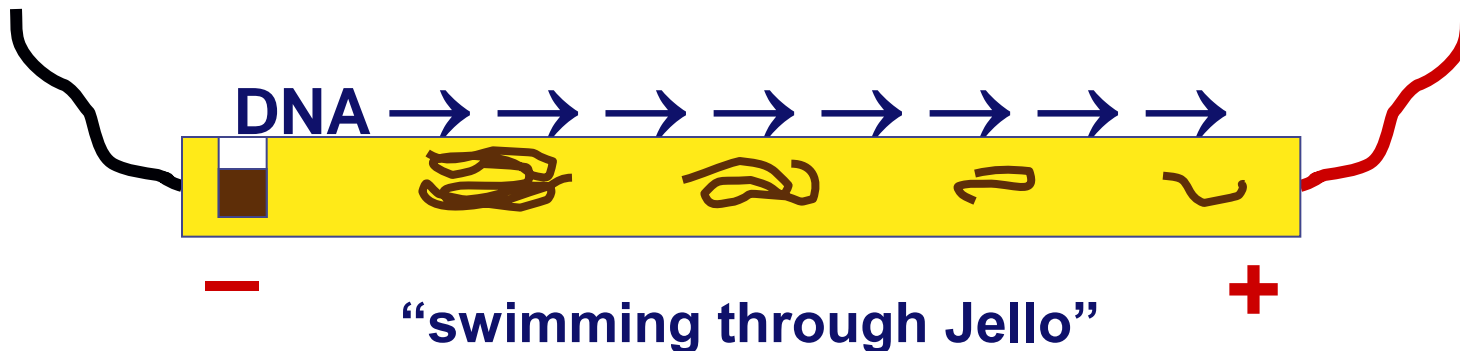
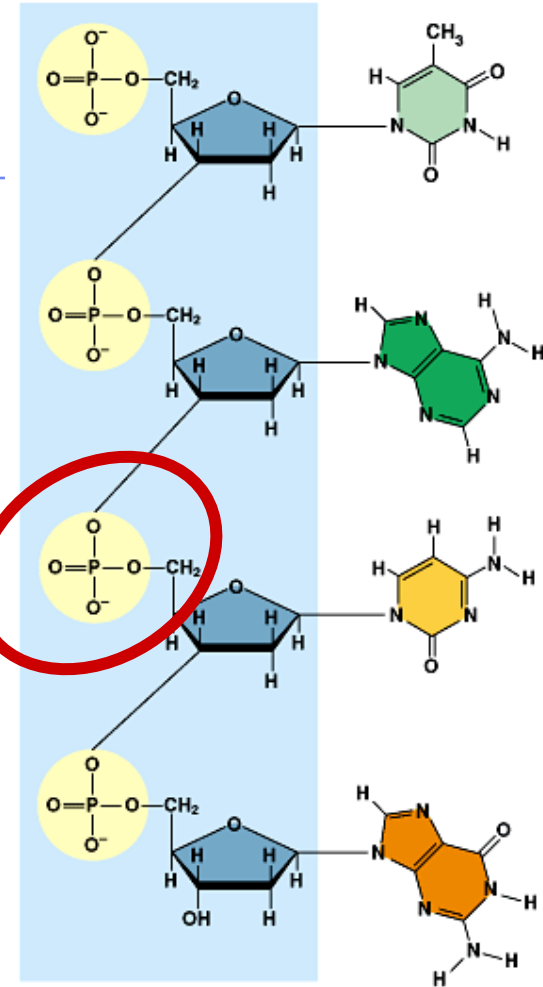
Comparing cut up DNA

- How do we compare DNA fragments?
 - ◆ separate fragments by size
- How do we separate DNA fragments?
 - ◆ run it through a gelatin
 - agarose
 - made from algae
 - ◆ gel electrophoresis



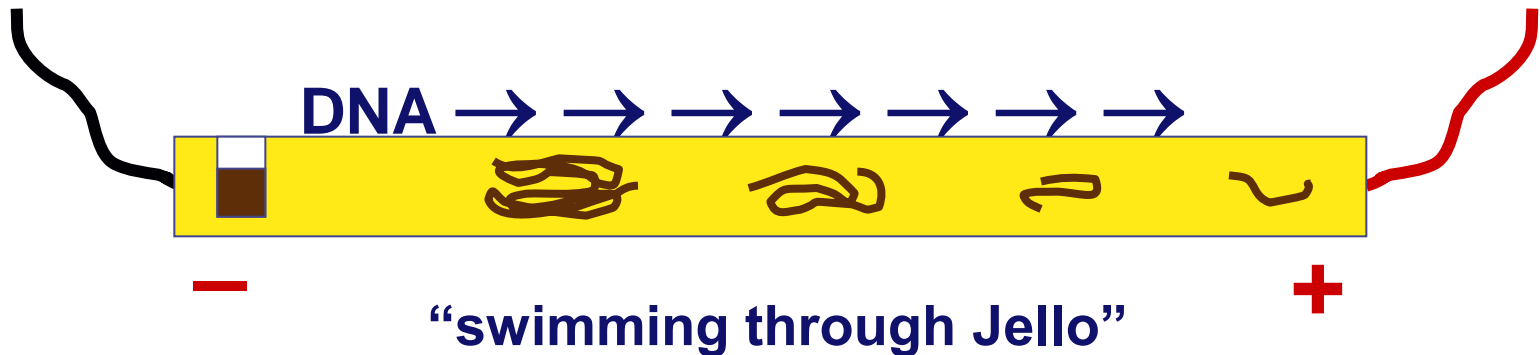
Gel electrophoresis

- A method of separating DNA in a gelatin-like material using an electrical field
 - ◆ DNA is negatively charged
 - ◆ when it's in an electrical field it moves toward the positive side



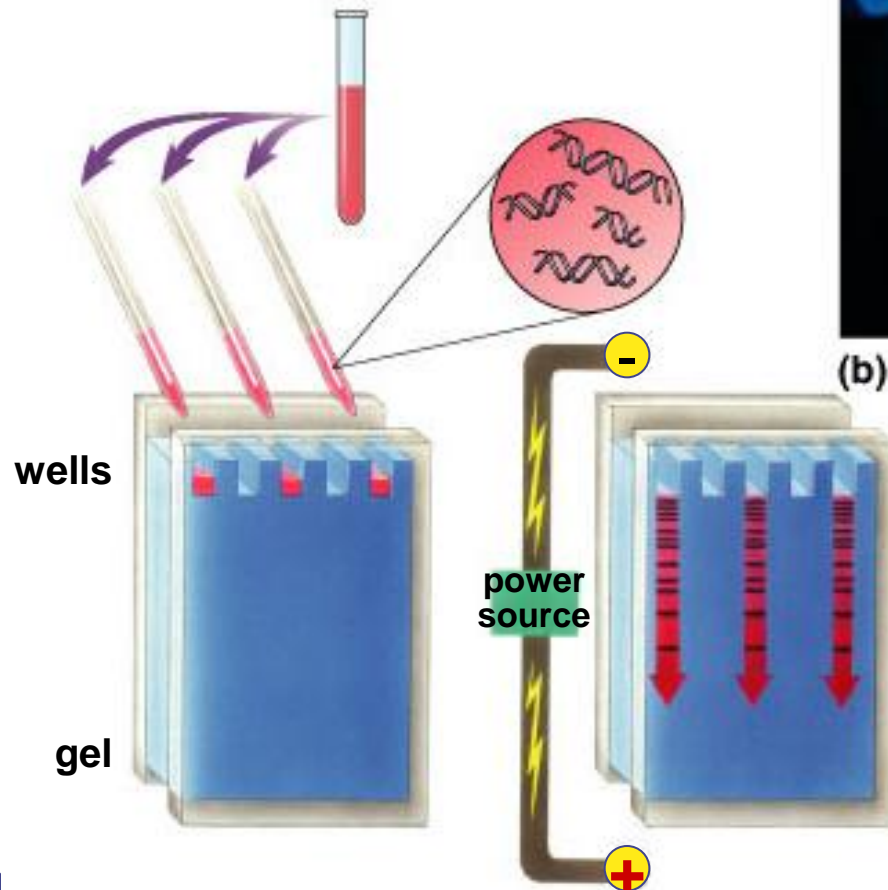
Gel electrophoresis

- DNA moves in an electrical field...
 - ◆ so how does that help you compare DNA fragments?
 - size of DNA fragment affects how far it travels
 - ◆ small pieces travel farther
 - ◆ large pieces travel slower & lag behind

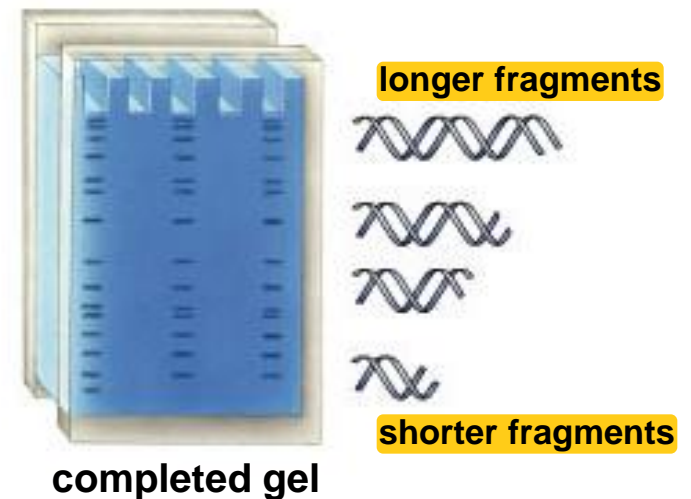


Gel Electrophoresis

DNA &
restriction enzyme

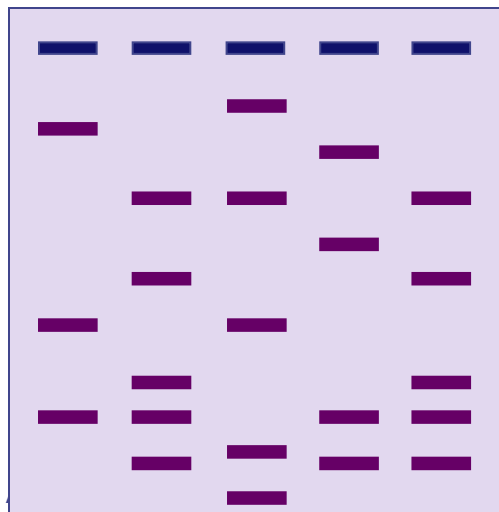
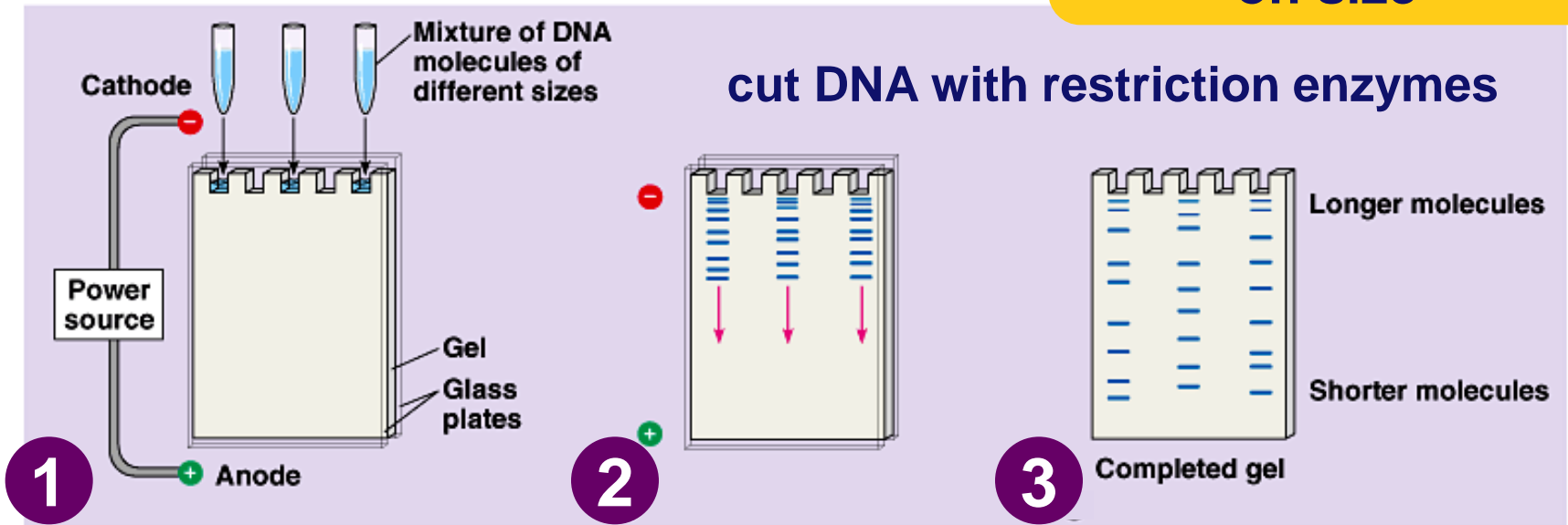


(b)



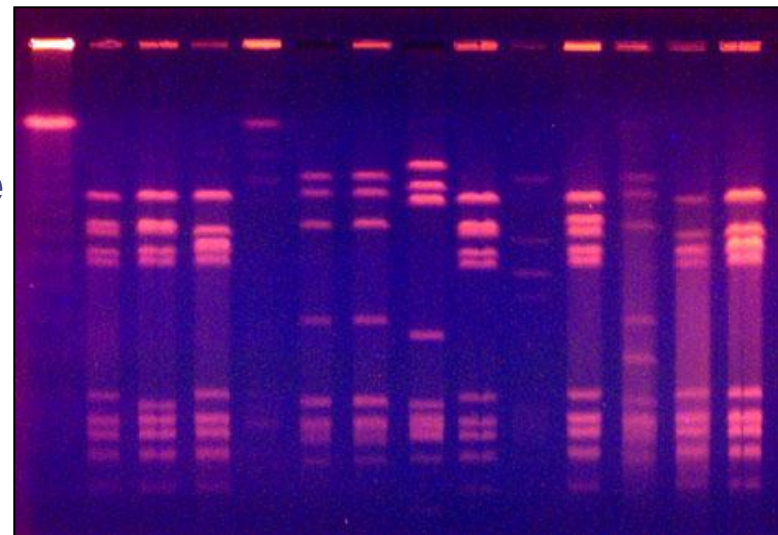
Running a gel

fragments of DNA
separate out based
on size



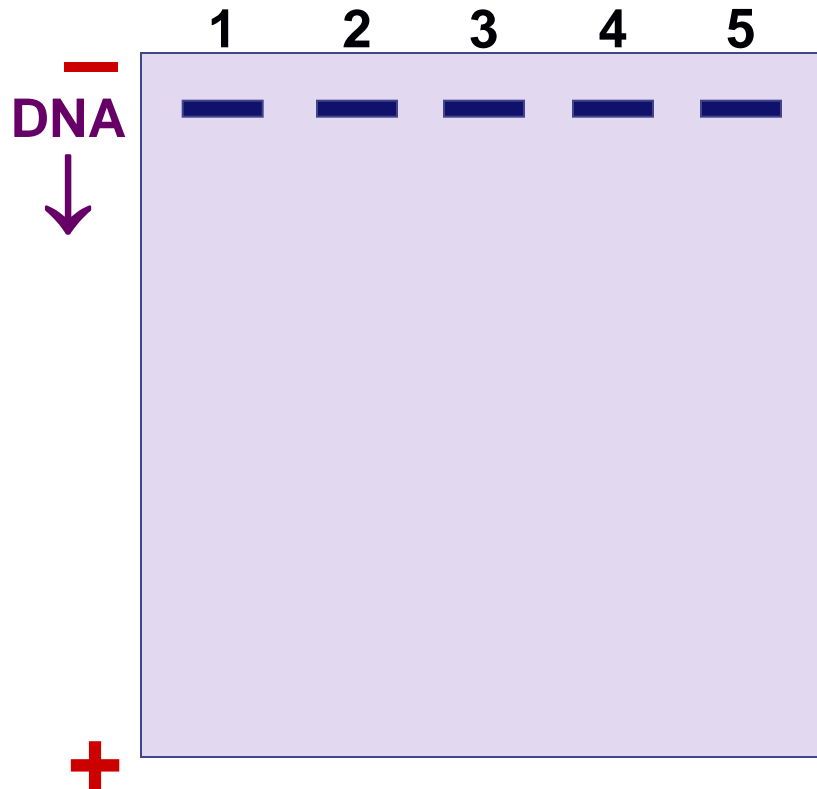
Stain DNA

- ◆ ethidium bromide binds to DNA
- ◆ fluoresces under UV light

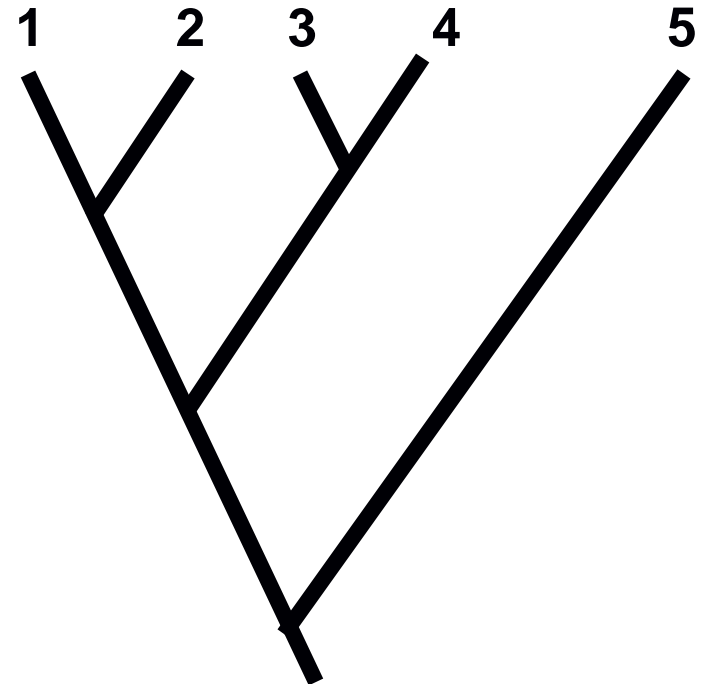


Uses: Evolutionary relationships

- Comparing DNA samples from different organisms to measure evolutionary relationships

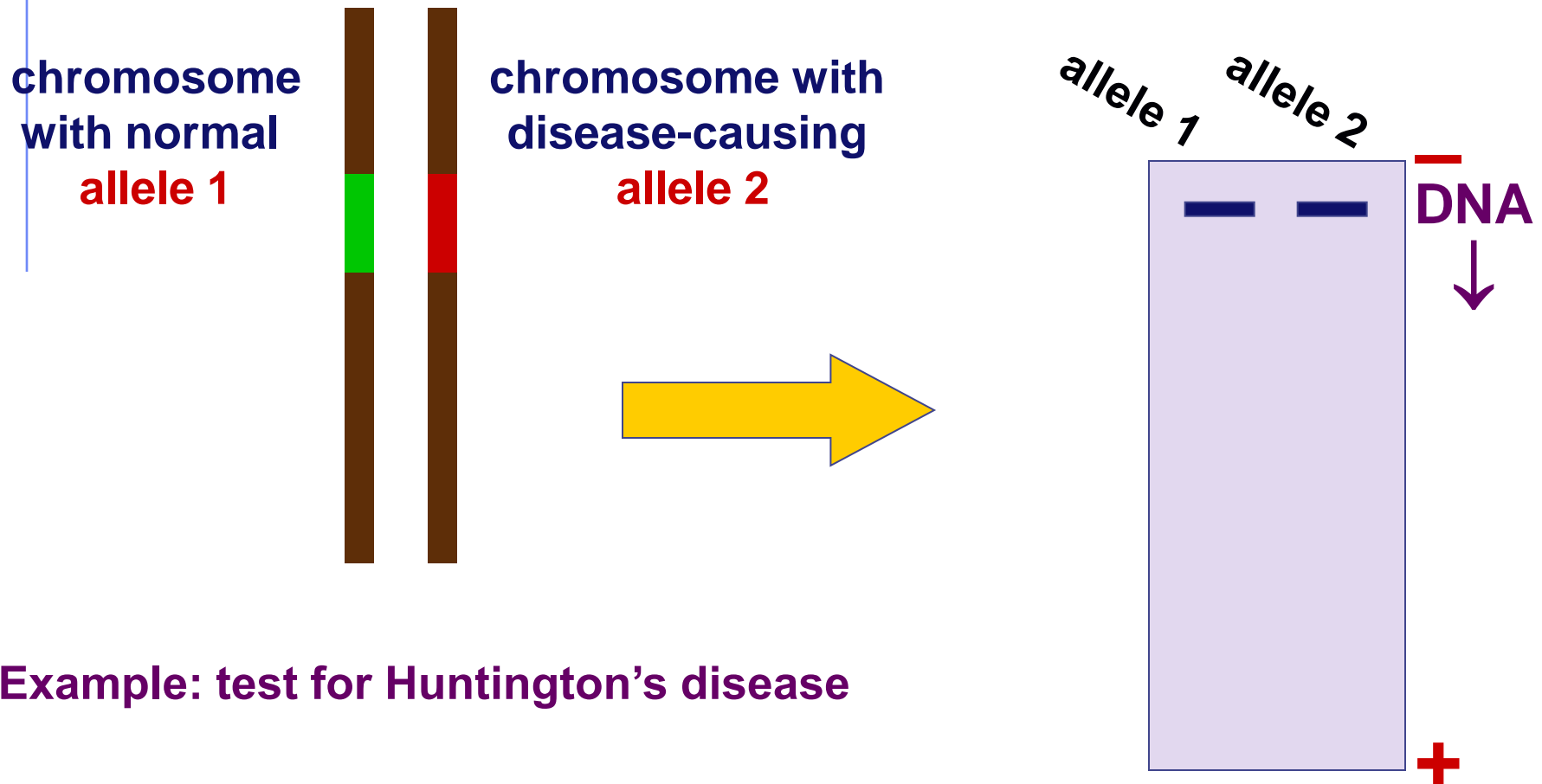


turtle snake rat squirrel fruitfly



Uses: Medical diagnostic

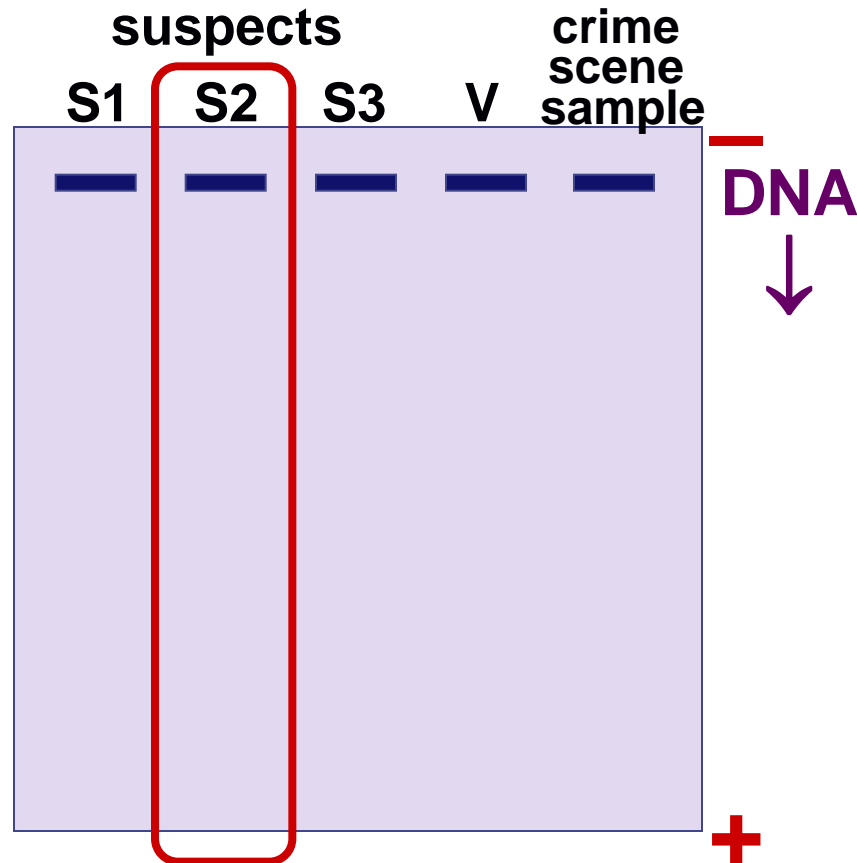
- Comparing normal allele to disease allele



Example: test for Huntington's disease

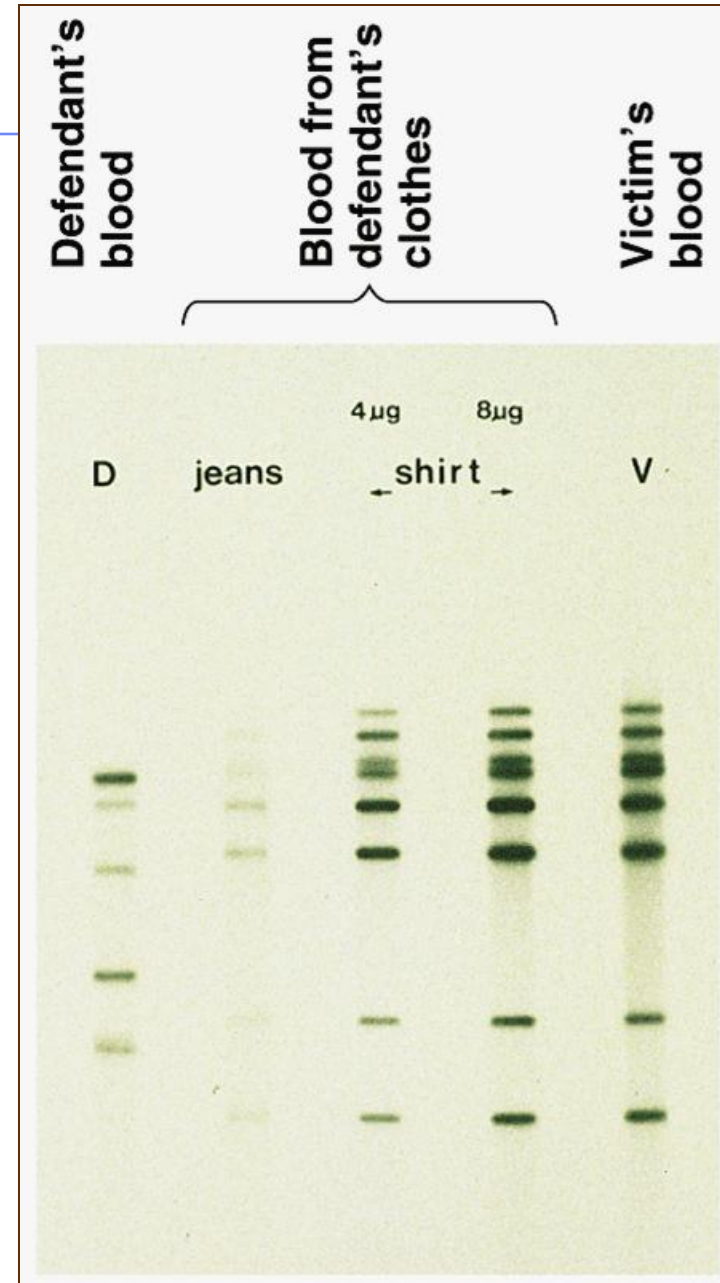
Uses: Forensics

- Comparing DNA sample from crime scene with suspects & victim



DNA fingerprints

- Comparing blood samples on defendant's clothing to determine if it belongs to victim
 - ◆ DNA fingerprinting
 - ◆ comparing DNA banding pattern between different individuals
 - ◆ ~unique patterns



Differences at the DNA level

- Why is each person's DNA pattern different?
 - ◆ sections of “junk” DNA
 - doesn't code for proteins
 - made up of repeated patterns
 - ◆ CAT, GCC, and others
 - ◆ each person may have different number of repeats
 - many sites on our 23 chromosomes with different repeat patterns

```
GCTTGTAACGGCCTCATCATCATTCGCCGGCCTACGCTT
CGAACATTGCCGGAGTAGTAGTAAGCGGCCGGATGCGAA
```

```
GCTTGTAACGGCATCATCATCATCATCCGGCCTACGC
CGAACATTGCCGTAGTAGTAGTAGTAGTAGGCGCGATGC
```

DNA patterns for DNA fingerprints

Allele 1

cut sites

repeats

cut sites

GCTTGTAACGGCCTCATCATCATTCGCCGGCCTACGCTT
CGAACATTGCCGGAGTAGTAGTAAGCGGGCCGGATGCGAA

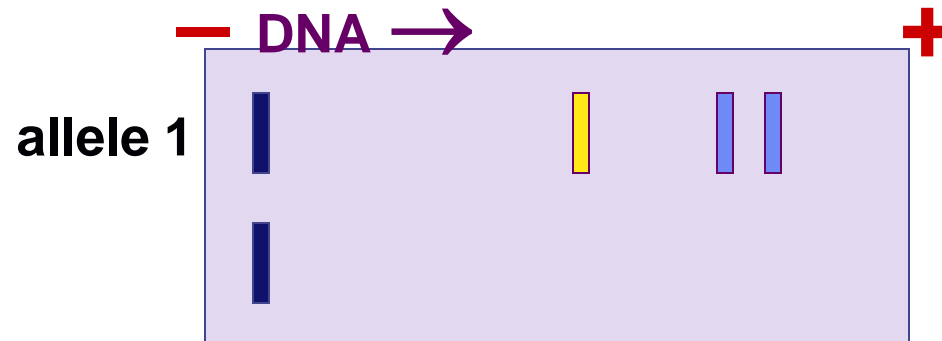
Cut the DNA

GCTTGTAACG G C C T C A T C A T C A T C G C C G G C C T A C G C T T
C G A A C A T T G C C G G A G T A G T A G T A G C G G G C C G G A T G C G A A

1

2

3



Differences between people

Allele 1

cut sites

cut sites

GCTTGTAACGGCCTCATCATCATTCGCCGGCCTACGCTT
CGAACATTGCCGGAGTAGTAGTAAGCGGCCGGATGCGAA

Allele 2: more repeats

GCTTGTAACGGCCTCATCATCATCATCATCCGGCCTA
CGAACATTGCCGGAGTAGTAGTAGTAGTAGTAGGCGCGGA

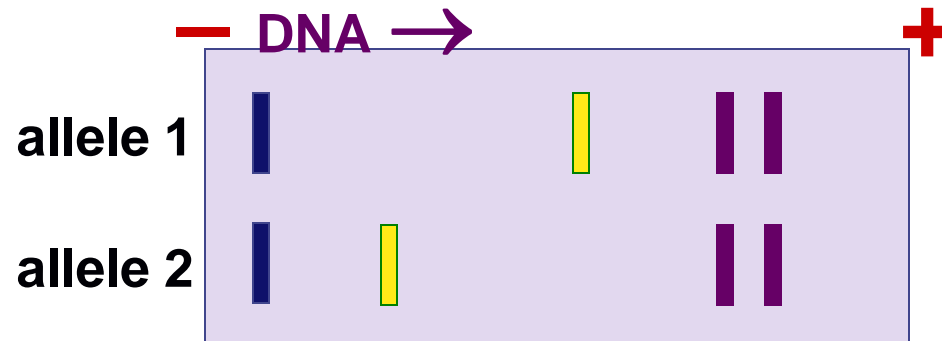
1

2

3

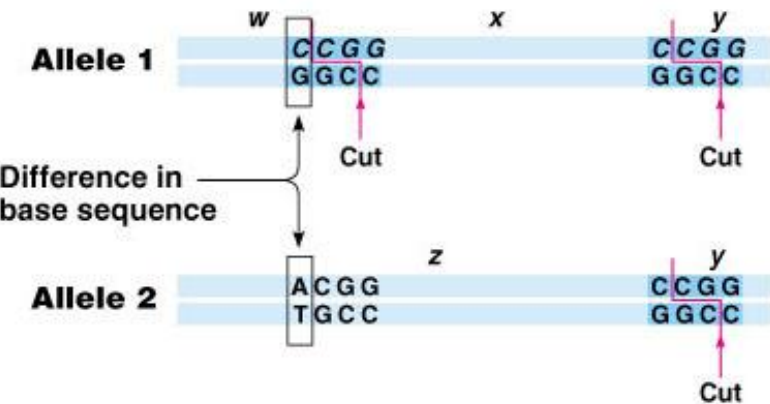


DNA fingerprint

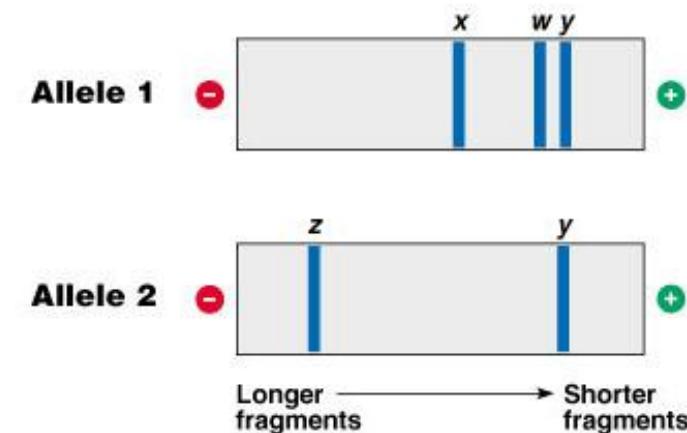


RFLPs

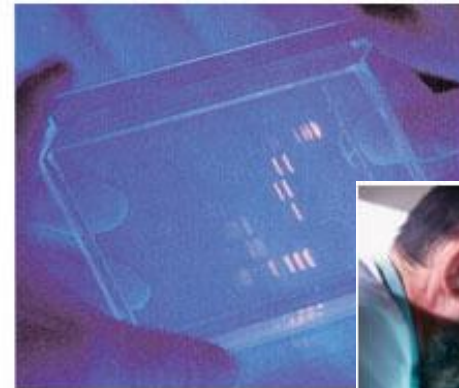
- Restriction Fragment Length Polymorphism
 - ◆ differences in DNA between individuals



(a) DNA from two alleles



(b) Electrophoresis of restriction fragments



(c) Completed gel

Alec Jeffries
1984

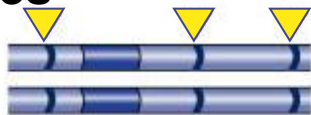


- ◆ change in DNA sequence affects restriction enzyme “cut” site
- ◆ creates different fragment sizes & different band pattern

Polymorphisms in populations

- Differences between individuals at the DNA level
 - ◆ many differences accumulate in “junk” DNA

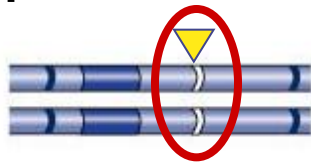
restriction enzyme
cutting sites



2 bands



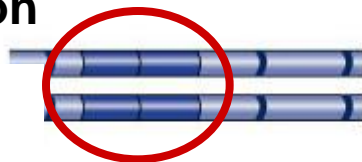
single base-pair
change



1 band



sequence
duplication



2 different bands



RFLP / electrophoresis use in forensics

- 1st case successfully using DNA evidence
 - ◆ 1987 rape case convicting Tommie Lee Andrews

“standard”

semen sample from rapist

blood sample from suspect

“standard”

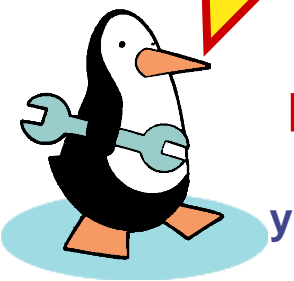
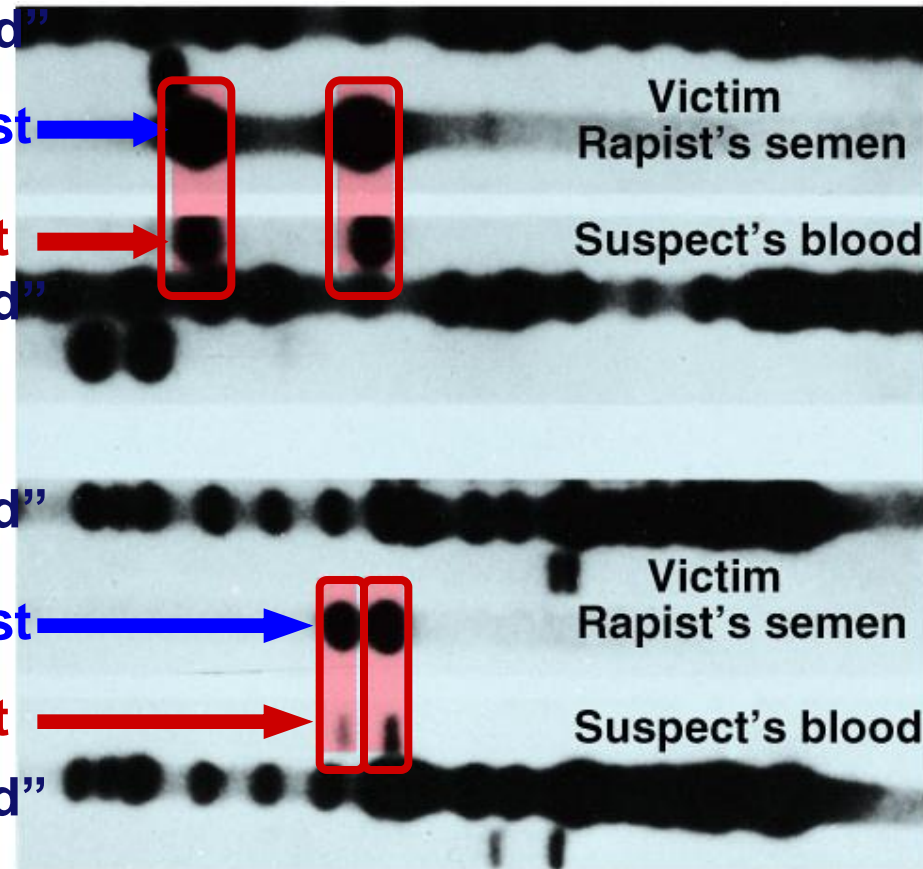
How can you
compare DNA from
blood & from semen?
RBC?

“standard”

semen sample from rapist

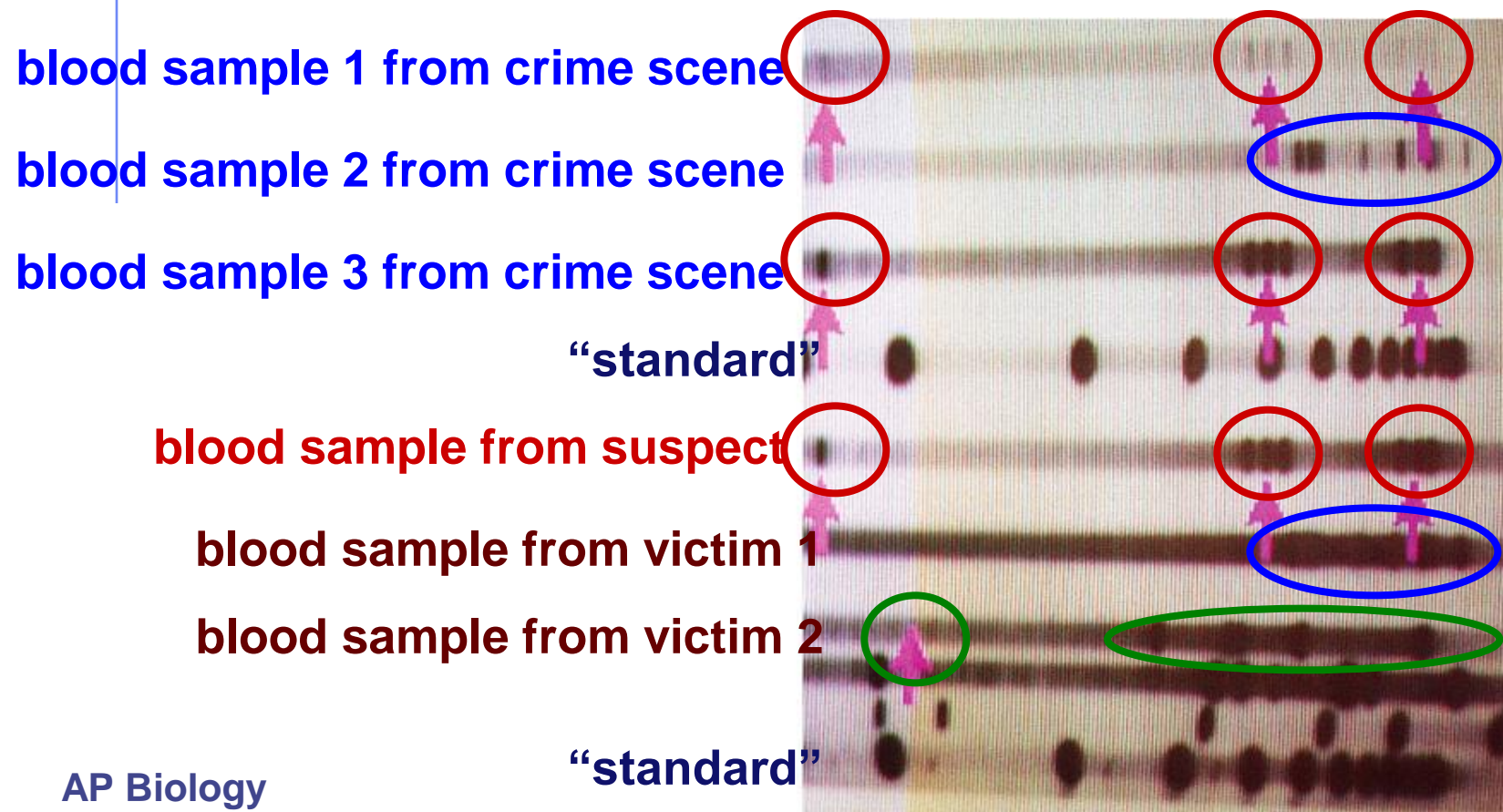
blood sample from suspect

“standard”



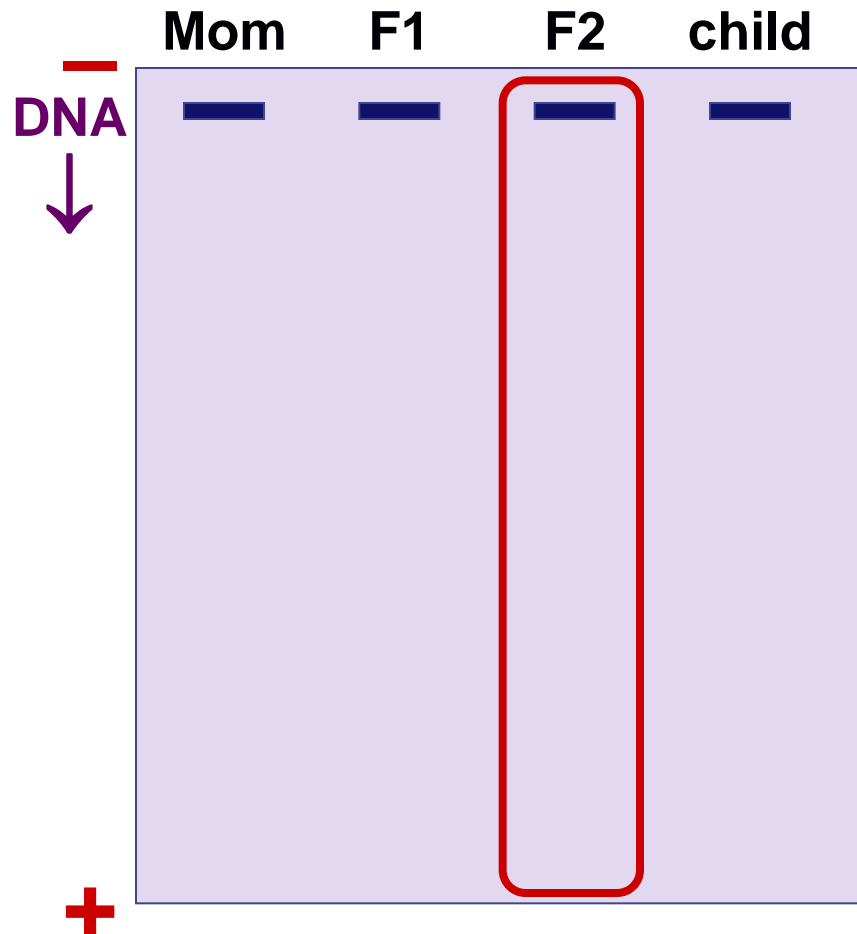
Electrophoresis use in forensics

- Evidence from murder trial
 - ◆ Do you think suspect is guilty?

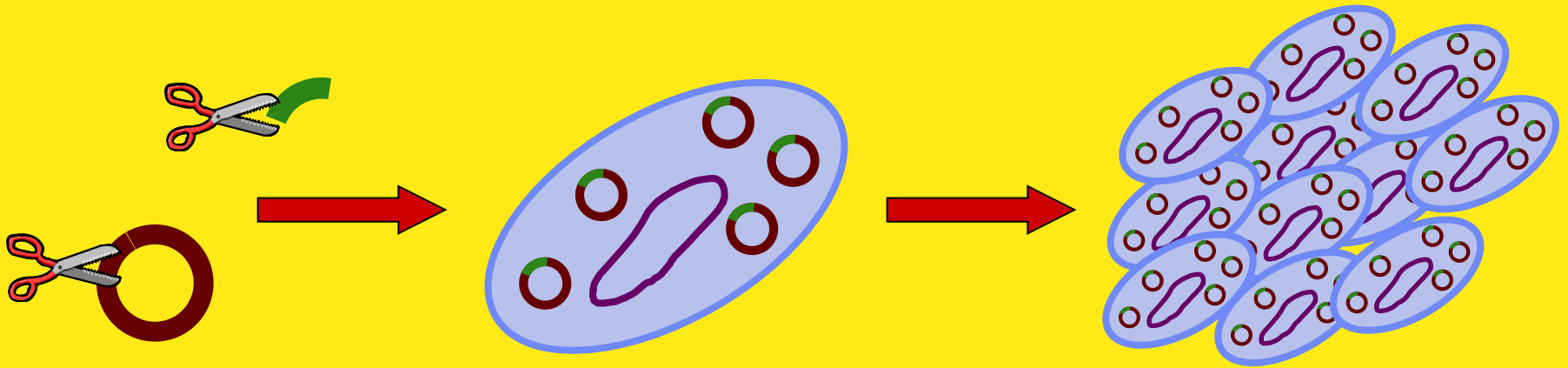


Uses: Paternity

- Who's the father?



Making lots of copies of DNA



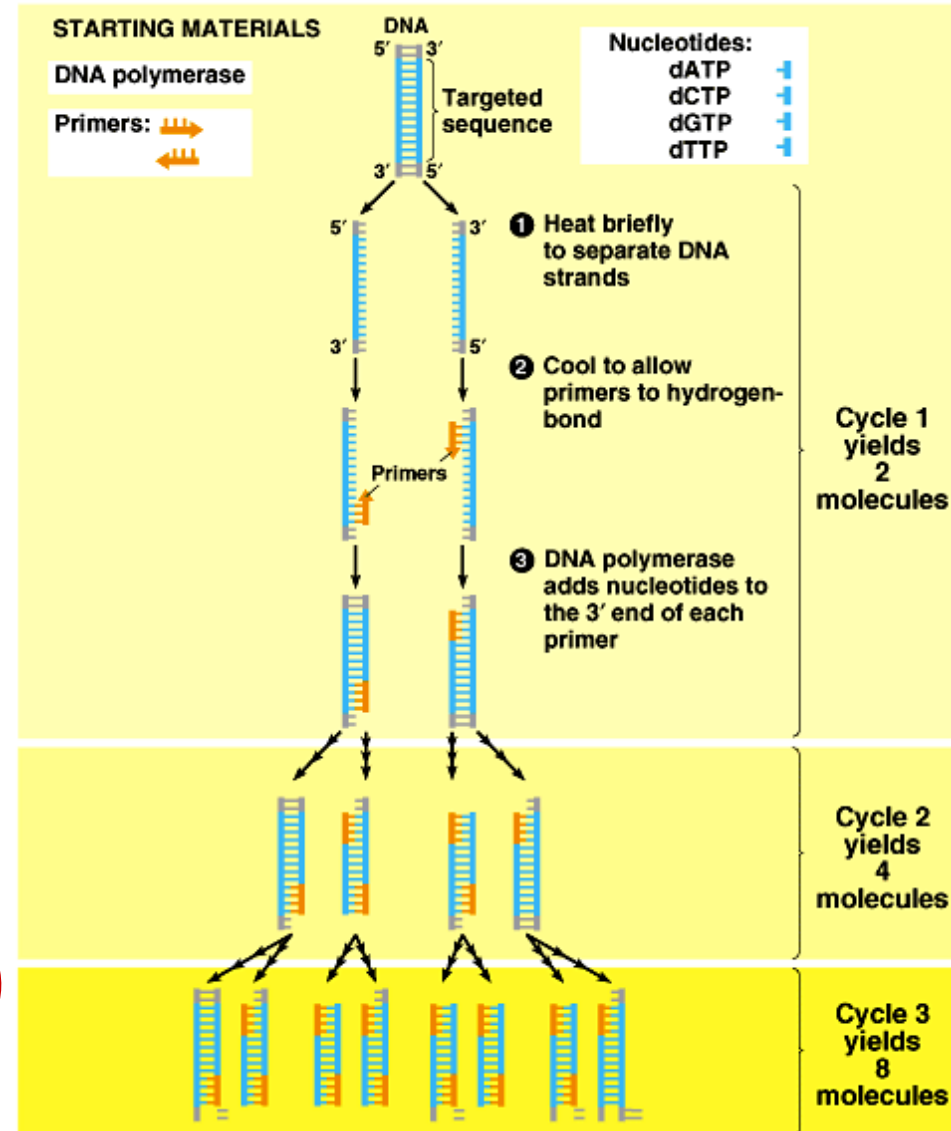
But it would be so much easier if we didn't have to use bacteria every time...

Copy DNA without plasmids? **PCR!**

■ Polymerase Chain Reaction

- ◆ method for making many, many copies of a specific segment of DNA
- ◆ ~only need 1 cell of DNA to start

No more bacteria,
No more plasmids,
No more E. coli
smelly looks!



PCR process

- It's copying DNA in a test tube!
- What do you need?
 - ◆ template strand
 - ◆ DNA polymerase enzyme
 - ◆ nucleotides
 - ATP, GTP, CTP, TTP
 - ◆ primer

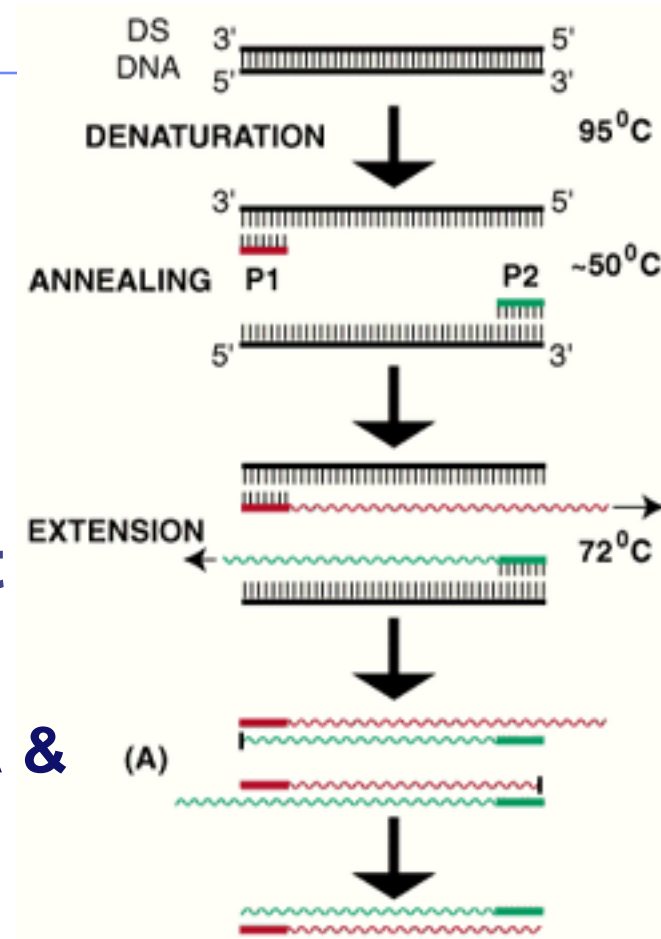


Thermocycler



PCR primers

- **The primers are critical!**
 - ◆ need to know a bit of sequence to make proper primers
 - ◆ primers can bracket target sequence
 - start with long piece of DNA & copy a specified shorter segment
 - primers define section of DNA to be cloned

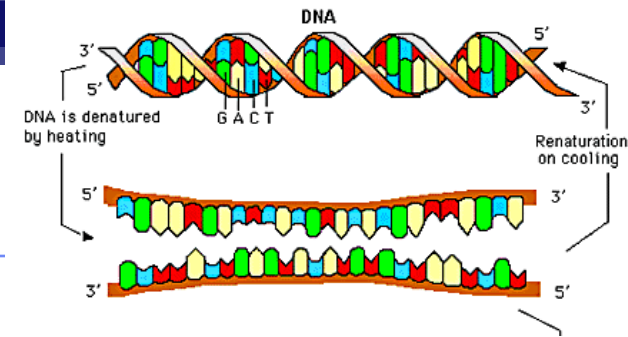


20-30 cycles
3 steps/cycle
30 sec/step

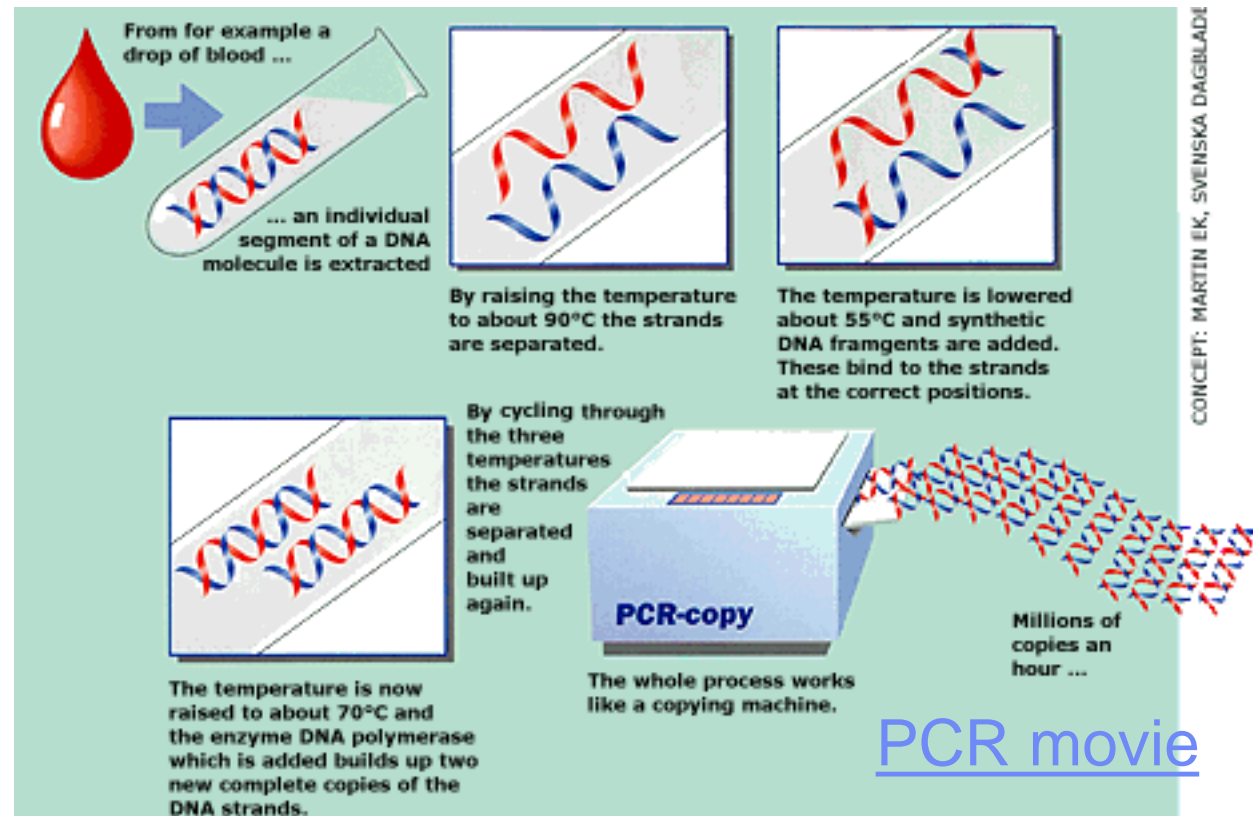
PCR process

■ What do you need to do?

- ◆ in tube: DNA, DNA polymerase enzyme, primer, nucleotides
- ◆ denature DNA: heat (**90°C**) DNA to separate strands
- ◆ anneal DNA: cool to hybridize with primers & build DNA (**extension**)



What does 90° C
do to our
DNA polymerase?



[PCR movie](#)

PCR

20-30 cycles

3 steps/cycle

30 sec/step

The polymerase problem

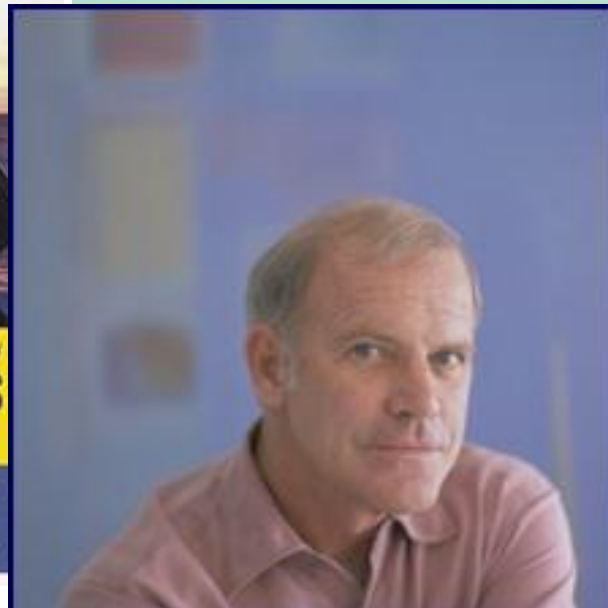
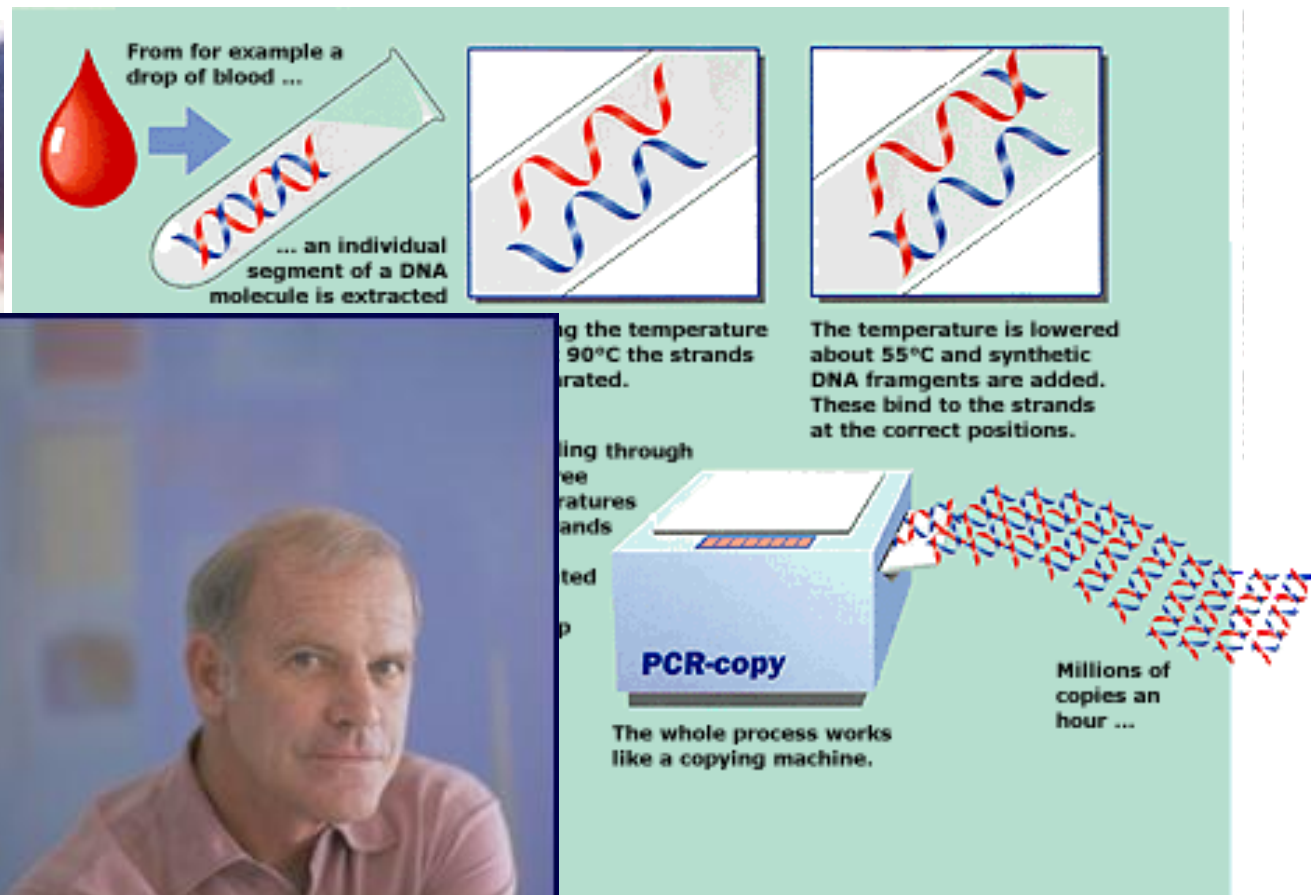
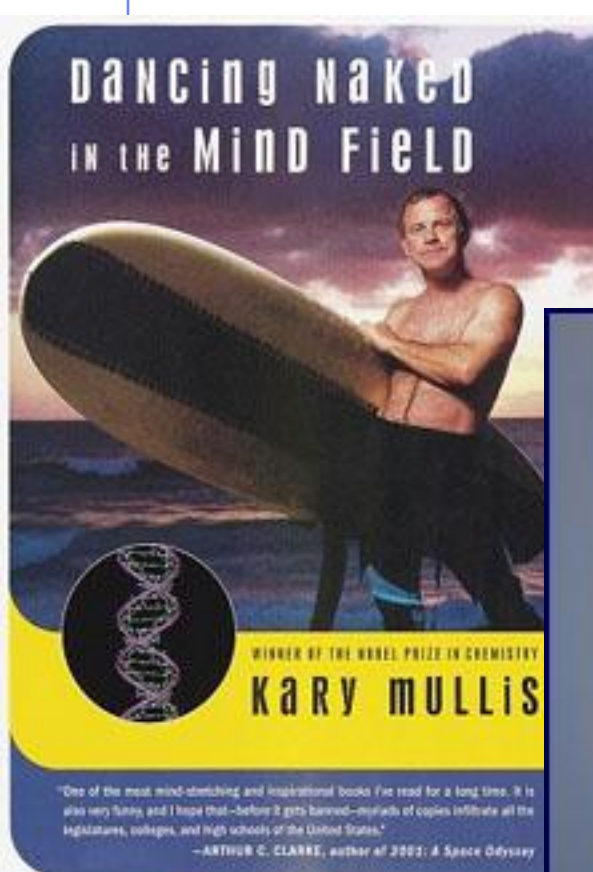
- Heat DNA to denature (unwind) it
 - ◆ 90°C destroys DNA polymerase
 - ◆ have to add new enzyme every cycle
 - almost impractical!
- Need enzyme that can withstand 90°C...
 - ◆ Taq polymerase
 - from hot springs bacteria
 - ◆ *Thermus aquaticus*



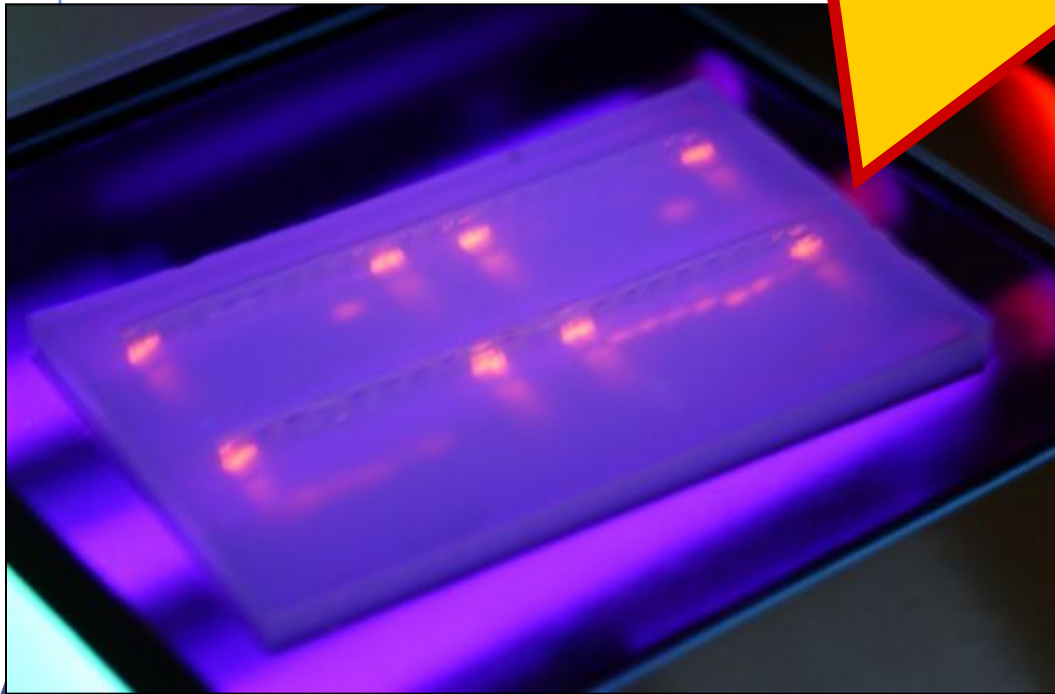
1985 | 1993

Kary Mullis

- development of PCR technique
 - ◆ a copying machine for DNA



I'm a-glow!
Got any Questions?



Gel Electrophoresis Results

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.