# Announcements

Wednesday– quiz on Chpt 1 of ECB (not Chpt 2)

HW #2, Due on Wednesday

Today: Applications of DNA:

FISH, DNA “chips”, Forensics

Next time: Fundamental studies:

bending & twisting rigidity of DNA with Magnetic Traps.

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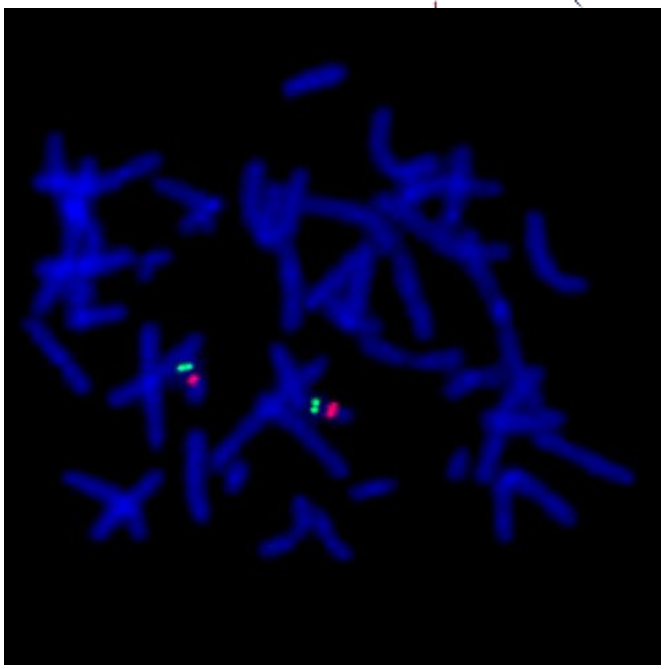
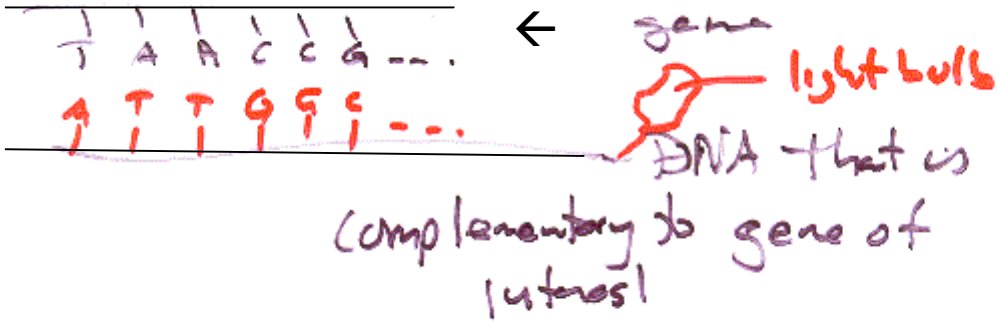
# The location of genes on a chromosome can be determined.

Technique called Fluorescence In Situ Hybridization (FISH)

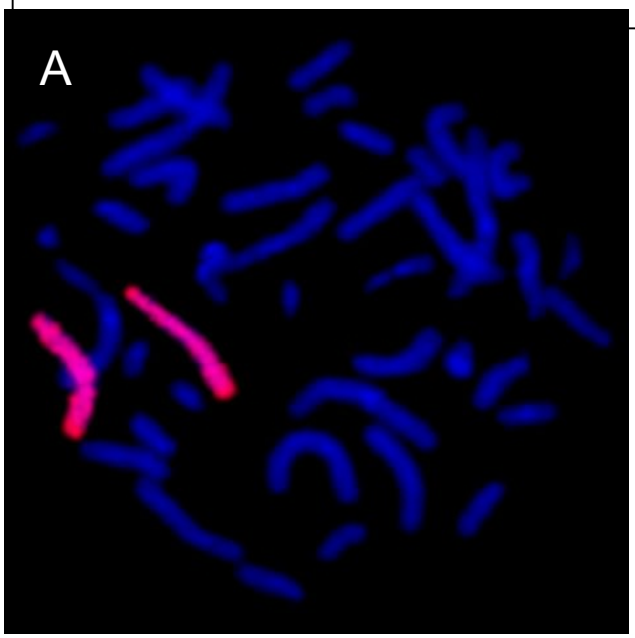


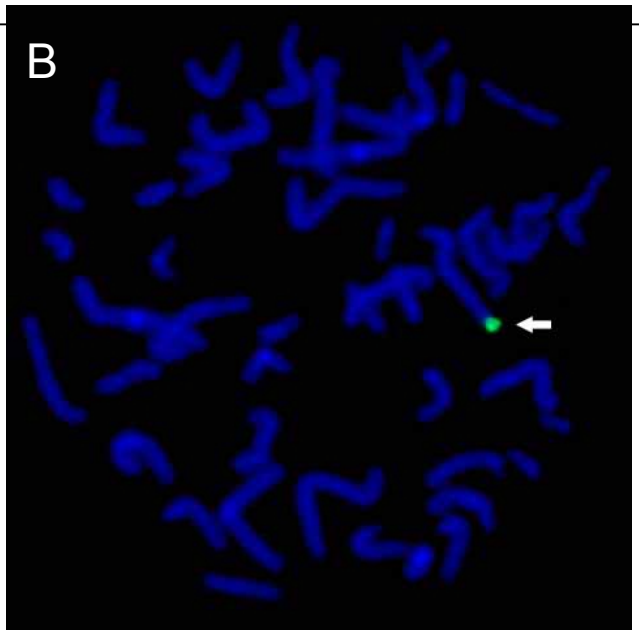

Green- # 22 marker- 22q13
Red- DiGeorge Syndrome region (if missing) at 22q11.2
(Person has 2→ normal)

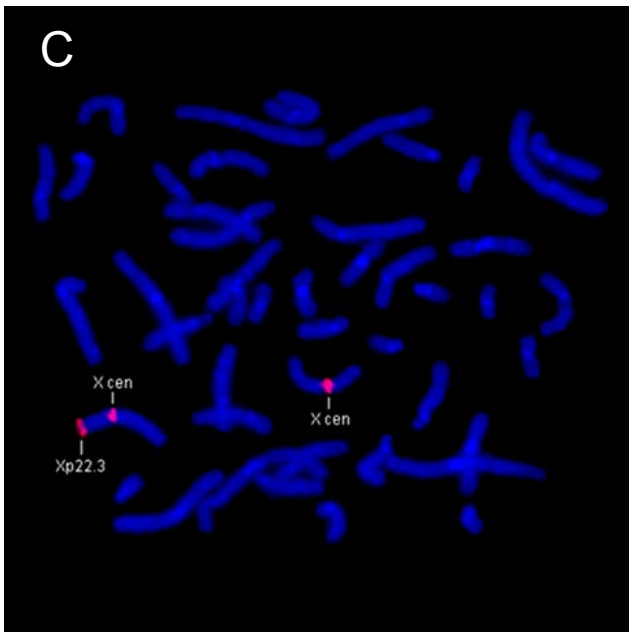



http://members.aol.com/chrominfo/metafish.htm

# FISH




A). Chromosone 4 “painted”.

B) From same person in A, but hybridized with a probe for the terminal part of chromosome 4q. Only one green signal → one chromosome 4 is missing material from the terminal end of 4q.

C) $X_{cen}$ → chromosome 22

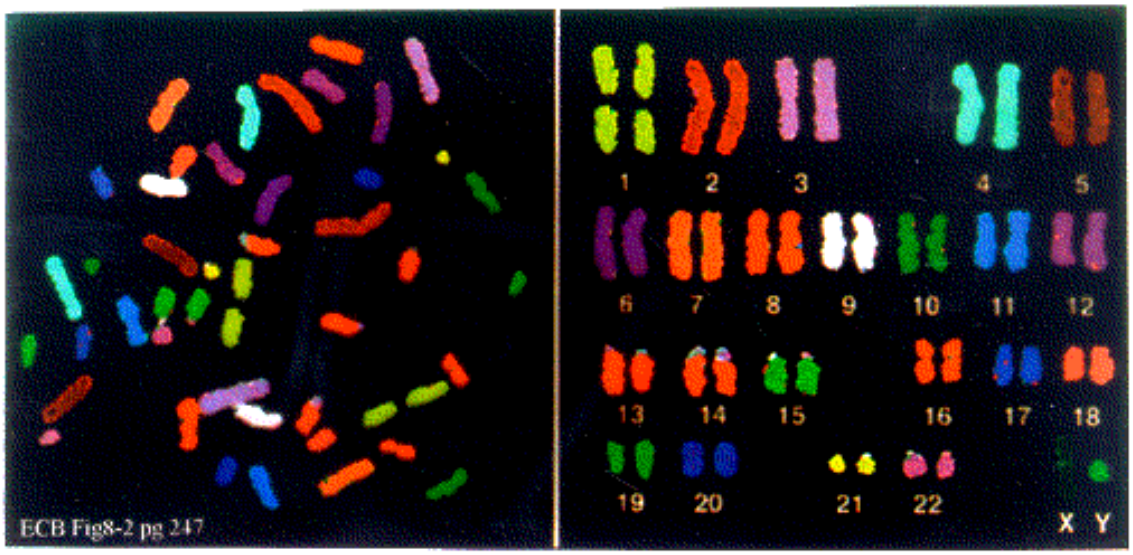
Other: Steroid Sulfatase gene

Two X chromosomes, 1 St.Su. gene → female carrier for Steroid Sulfatase Deficiency.

http://members.aol.com/chrominfo/metafish.htm

# (Mitotic) Chromosomes can be identified by their unique sequences

Fluorescently tagged DNA complementary to these unique sequences are used as markers.




Technique called "Karyotyping"

**Clinical uses: test for chromosomal abnormalities**

e.g. Trisomy 21 = "Down's syndrome"

## Central Dogma of Molecular Biology

### From DNA to RNA to proteins

Replication

DNA → RNA → Proteins

DNA polymerase

RNA polymerase

Ribosome

DNA → DNA: Replication

DNA → RNA: Transcription DNA+RNA same alphabet

RNA → Protein: Translation. alphabet of nucleotides, A,T.. to diff. " a.a.'s

# Modification of Dogma

## Reverse Transcriptase: ssRNA→ ssDNA

Replication

DNA ← → RNA → Proteins

DNA polymerase

RNA polymerase

Ribosome

Temin & Baltimore in 1970:1975 Nobel Prize

Important example of this? a) HIV-AIDS viral infection b) Telomerase (ends of chromosomes) R.T. (normal process)

Replicates "poorly": lots of errors.
1 per 15-30 kbases; compared to 1 in $10^9$ for DNA polymerase
No proofreading, unlike DNA polymerase.
AIDS uses this cleverly. How?

# Exploring the new world of the genome with DNA microarrays

Patrick O. Brown[1,3] & David Botstein[2]

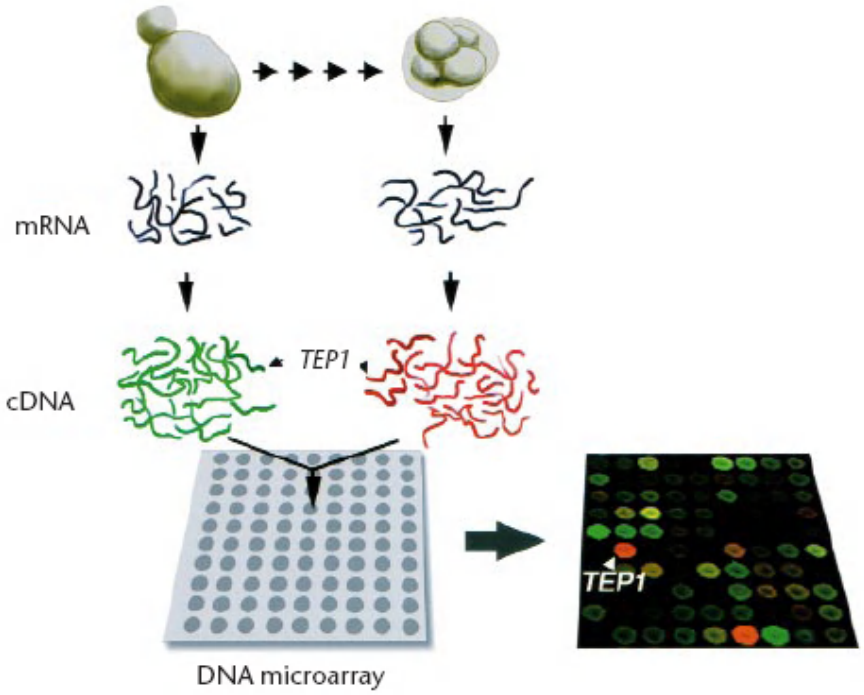
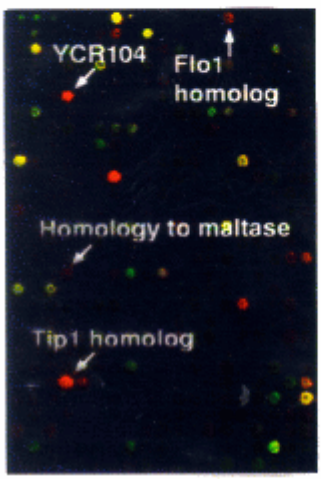


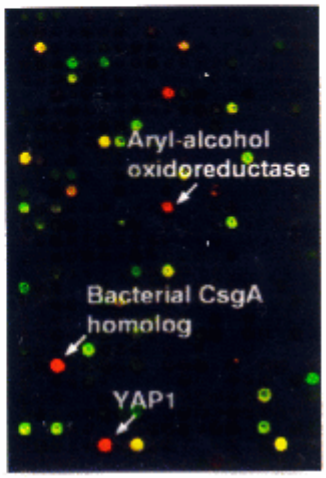

**Fig. 1** Gene expression analysis using a DNA microarray. In this illustration, mRNA samples from vegetative and sporulating yeast cells are compared. The total pool of messenger RNA from each cell population is used to prepare fluorescently labelled cDNA by reverse transcription in the presence of fluorescently labelled nucleotide precursors. To allow direct comparison of the abundance of each gene in the two samples, the two samples are labelled with different fluors—in this example, a red fluor for the mRNA from sporulating yeast and a green fluor for the mRNA from the vegetative yeast cells. The two fluorescently labelled cDNAs are then mixed and hybridized with a DNA microarray in which each yeast gene is represented as a distinct spot of DNA. Irrespective of their fluorescent labels, the cDNA sequences representing each individual transcript hybridize specifically with the corresponding gene sequence in the array. Thus, the relative abundance in sporulating as compared with vegetative yeast cells of the transcripts from each gene is reflected by the ratio of 'red' to 'green' fluorescence measured at the array element representing that gene. For example, the greater relative abundance of the *TEP1* mRNA in the sporulating cells results in a high ratio of red-labelled to green-labelled copies of the corresponding cDNA, and an equivalent ratio of red to green signal hybridized at the array element composed of DNA from *TEP1*.

*Nature Genetics Supplement* 21:33-37 (January 1999) (PDF 1M)

## Gene Arrays ("Chips") can be made
## Gene expression (i.e. RNA) can be detected on genome-wide scale : revolution!!

(Non-fluorescent) genes put on chip at defined position.

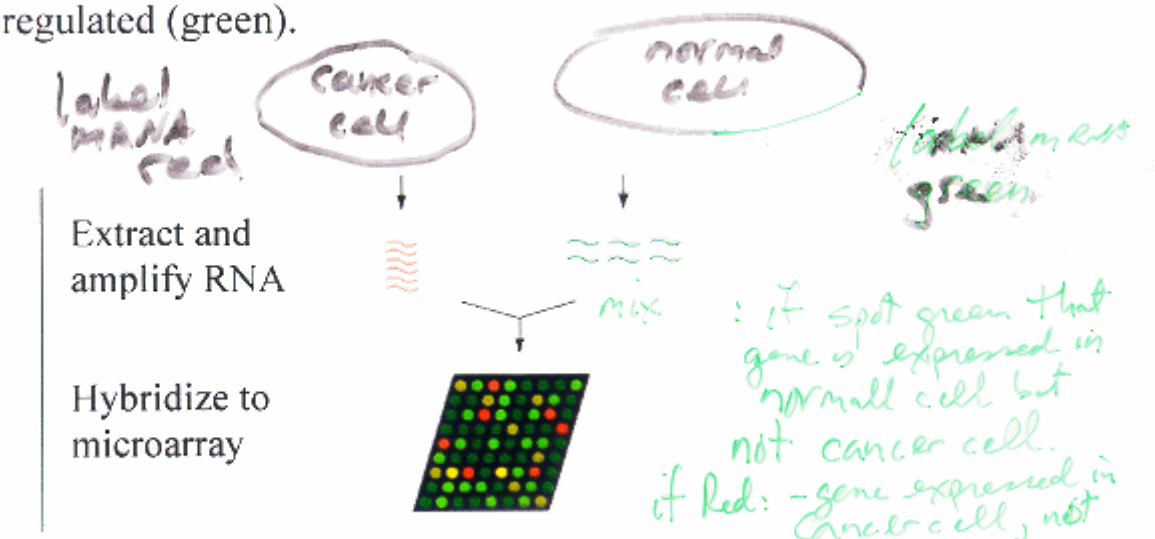



2 procedures

1. RNA from cell of interest extracted and fluorescently labeled e.g. with red dye. Added to chip, hybridized. Unbound washed away. Which position lights up tells which gene is active in cell!

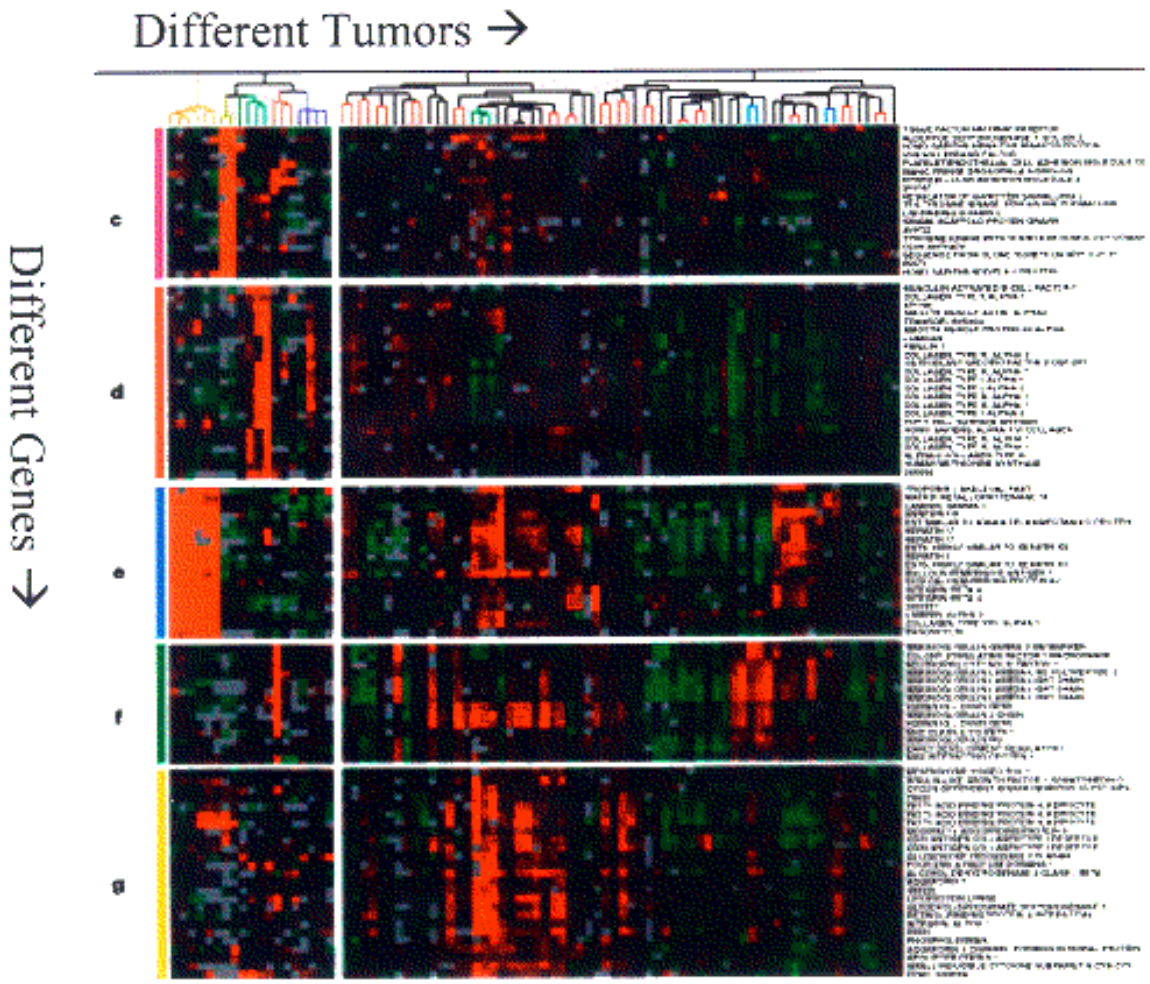
2. By using red from "normal" cell, green from test cell, can tell which genes are up-regulated (red) and which are down-regulated (green).



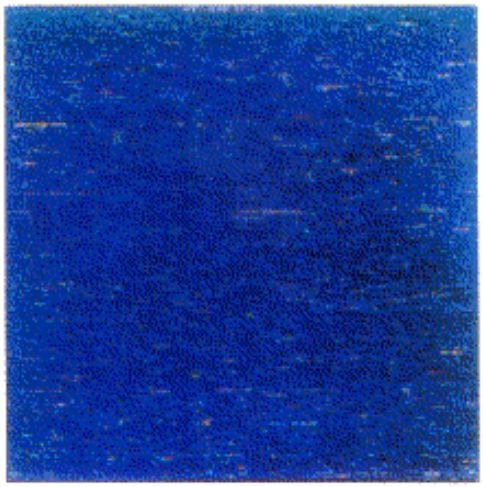

: if spot green that gene is expressed in normal cell but not cancer cell.
if Red: - gene expressed in cancer cell, not normal cell
Yellow - both cells express

## Gene chips can be used to follow genetic changes during cancer (and cancer treatment).

Variation in expression of 1,753 genes in 84 breast cancer samples. Data are presented in a matrix format: each row represents a single gene, and each column an experimental sample. Green squares, transcript levels below the median; black squares, transcript levels equal to the median; red squares, transcript levels greater than the median; grey squares, technically inadequate or missing data. From Perou et al, *Nature* **406**, 747 (2000).



# Gene chips can be used to follow genetic changes during development…



Different spots light up, i.e. genes turned on,
at different times (developmental stages) in life cycle.

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# DNA Forensics

Every person has their own, unique DNA (except for twins).

A person can be “tagged” with their DNA.

If your blood, semen is found→ you’re in trouble.

Your genes found in a kid → parent.

You’re killed in war/car accident and can only recognize you from your DNA.

Don’t have to completely sequence their DNA.
Can find certain regions. Just enough –say 13 different ones– that chances that another person has exactly the same set is 1 in a trillion.

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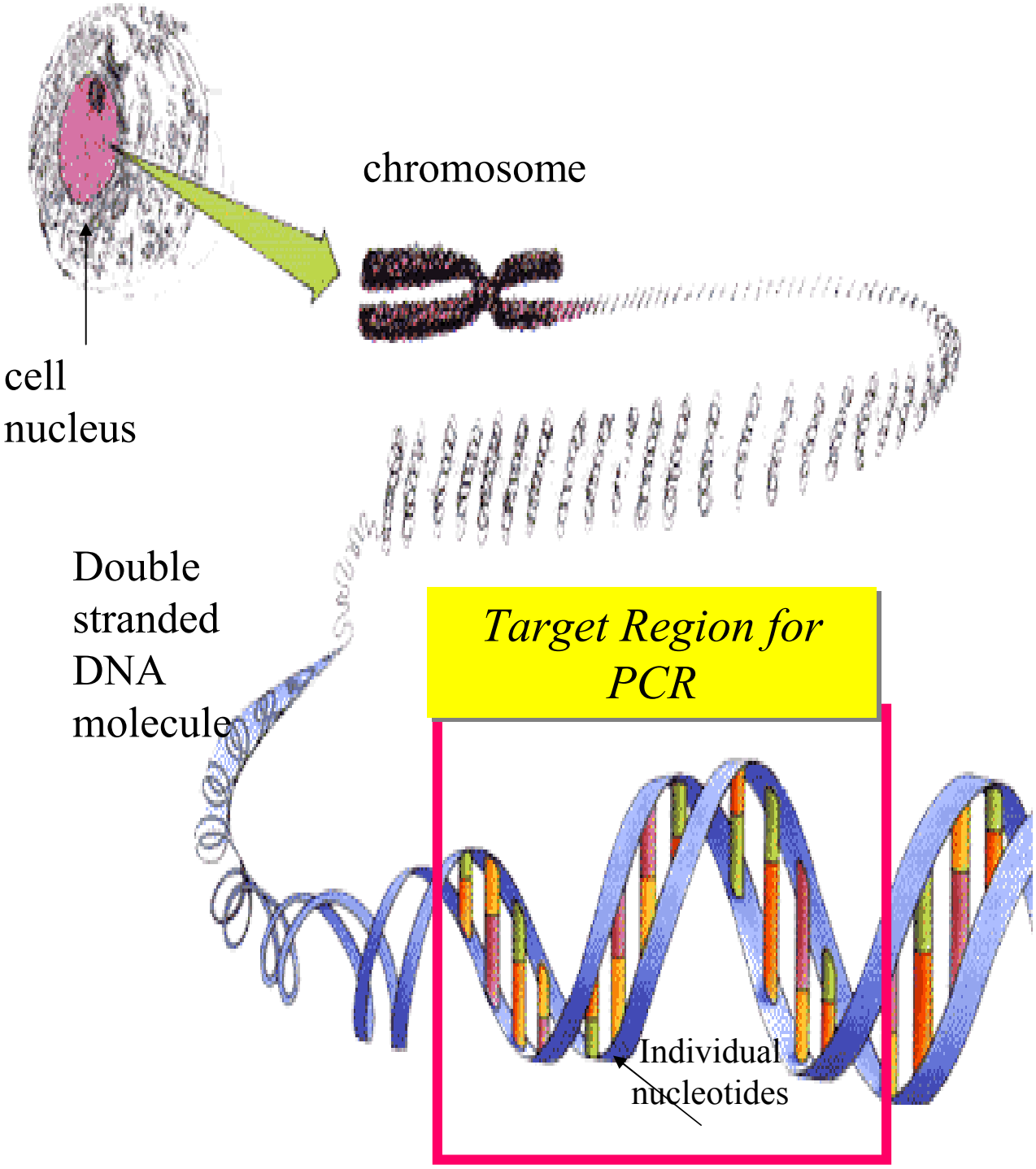
# DNA in the Cell




PCR, stands for? Polymerase Chain Reaction what is it?

Invented 1990; Nobel Prize in 1993: Kary Mullis

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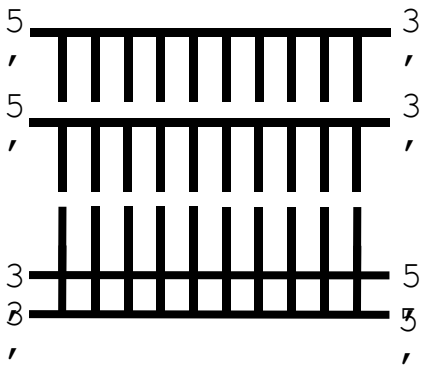
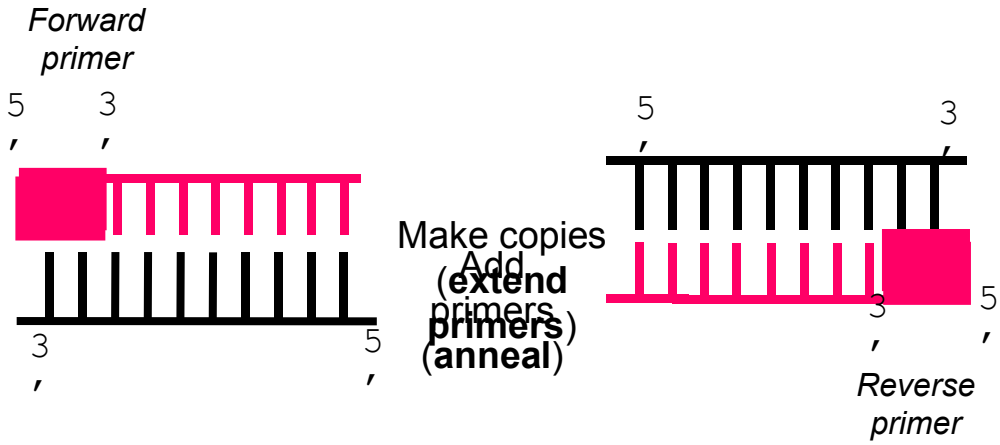
# DNA Amplification with the Polymerase Chain Reaction (PCR)




*Starting DNA Template*

Separate strands (**denature**)



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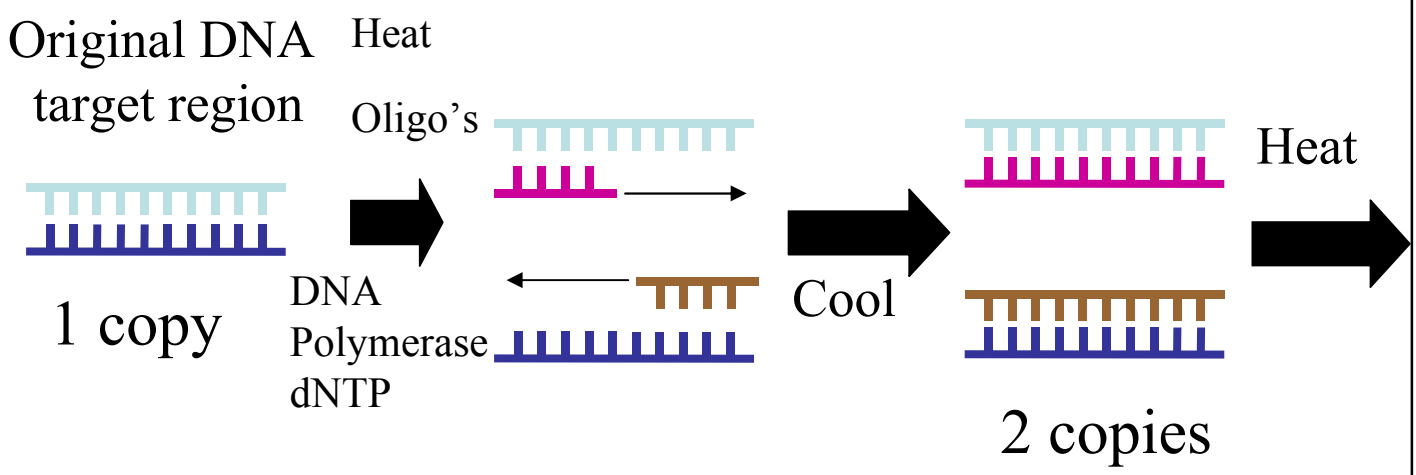
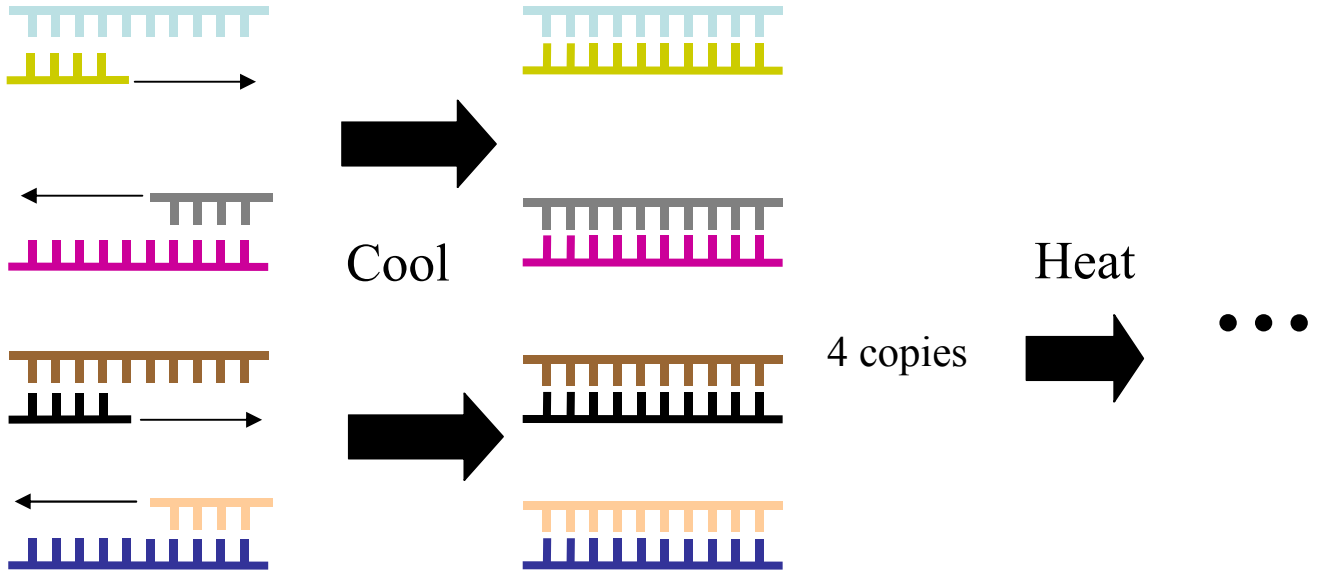
## PCR (Polymerase Chain Reaction) Copies DNA Exponentially through Multiple Thermal Cycles




*In 32 cycles at 100% efficiency,* ***1.07 billion*** *copies are created*

To work, what property of DNA polymerase have to have?

**New Scientists (1998)…Yellowstone's bugs** land up in court **...** Microorganisms from **hot** springs are especially valuable because their enzymes are not easily destroyed by heat. **...**

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# Forensic DNA Typing

or

## Did you kill (rape, father…) that person?

How DNA can "definitively" say.

*Adapted from:*

National Institutes of Science & Technology

http://www.cstl.nist.gov/div831/strbase/intro.htm

# DNA Use in Forensic, Paternity… Cases

- Most Forensic cases are rape cases (>2 out of 3)
  Looking for match between evidence and suspect
  -- **matching suspect with evidence**
- Paternity testing -- **identifying father**
- Military DNA "dog-tag."

## Challenges

•Mixtures must be resolved

•DNA is often degraded (stored wet- have mold, nuclease)

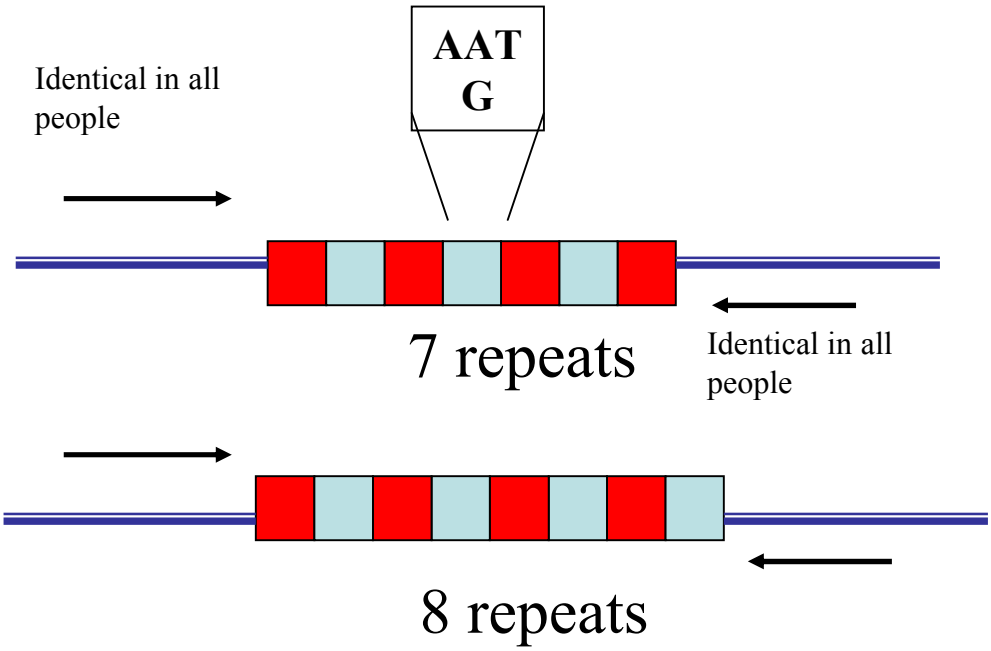
•Inhibitors to PCR are often present

# Sources of Biological Evidence

- **Blood**
- **Semen**
- **Saliva**
- **Urine**
- **Hair**
- **Teeth** (useful in fires).
- **Bone** (Yes, there are cells in bone. Decalcify it. 100,000 year old people, Dinosaurs- has DNA!)
- **Tissue**

**All felony arrests- cheek swab.**

# Short Tandem Repeats (STRs) (say chromo 3)




***the repeat region is variable between samples while the flanking regions where PCR primers bind are constant***

Homozygote = both alleles are the same length

Heterozygote = alleles differ and can be resolved from one another

# Variation Among STRs

Choosing which STRs:
Significant statistical variation – but not too many. Freq. that are measured in pop. : Loc 1 -10%. Loc 2 – 10%; locus 1+2 -1/100.
Random match with 13 primers (used now): $1/10^{13}$.
(There are 6 billion people, ~ $6 \times 10^9$ people.)
Watch out for different racial types!

# Multiplex PCR

- **Over 10 Markers Can Be Copied at Once**
- **Sensitivities to levels less than 1 ng of DNA**
- **Ability to Handle Mixtures and Degraded Samples**
- **Different Fluorescent Dyes Used to Distinguish STR Alleles with Overlapping Size Ranges**

Most rxns: require 2 PCR (tubes) 7 or 8 primer pairs in one tube– need total of about 2 tubes for 13 different STRs.

$20-$25 per rxn in lab. $150 incl labor. Cost for forensic up to $1000.

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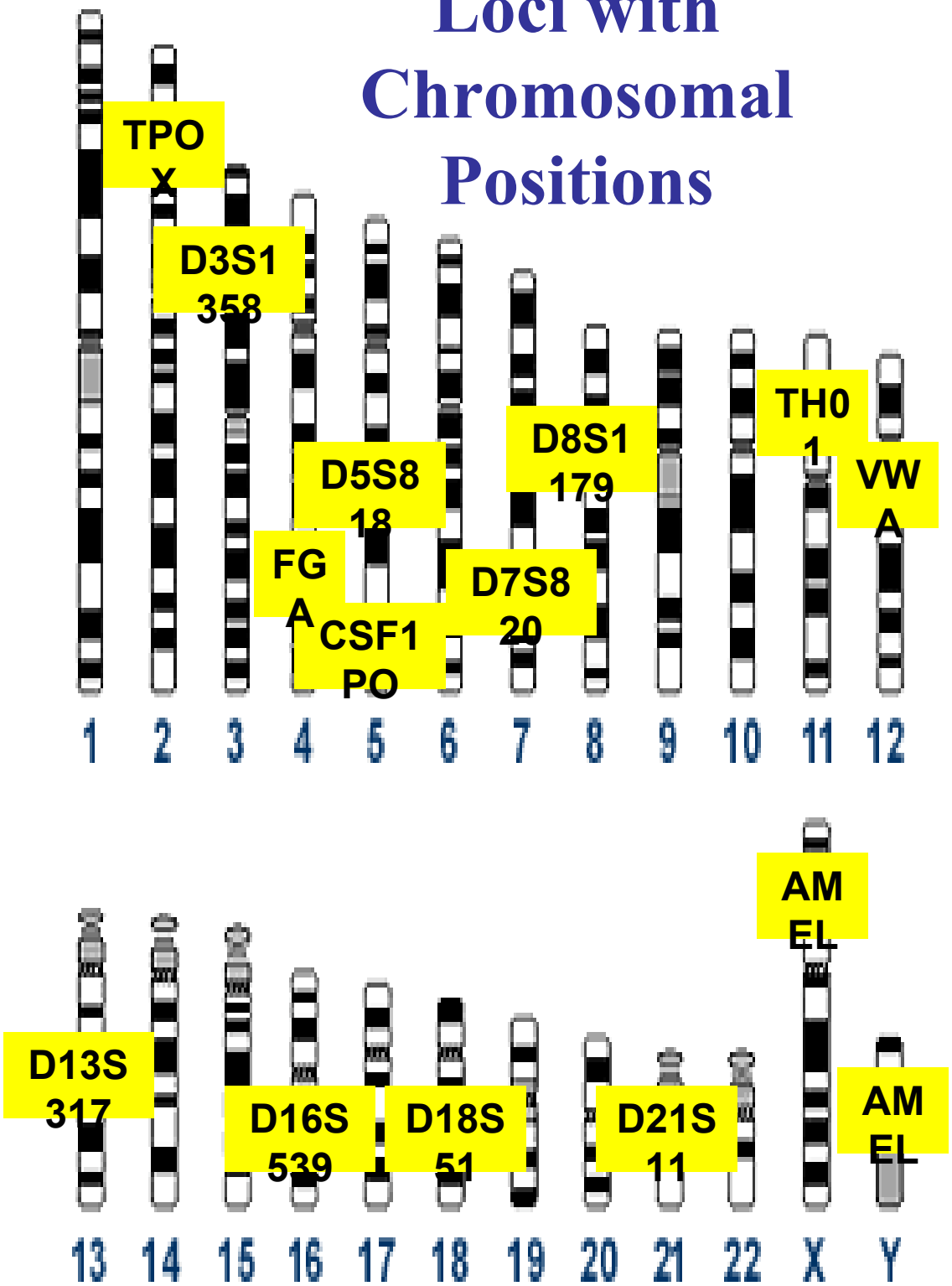
# 13 CODIS Core STR Loci with Chromosomal Positions



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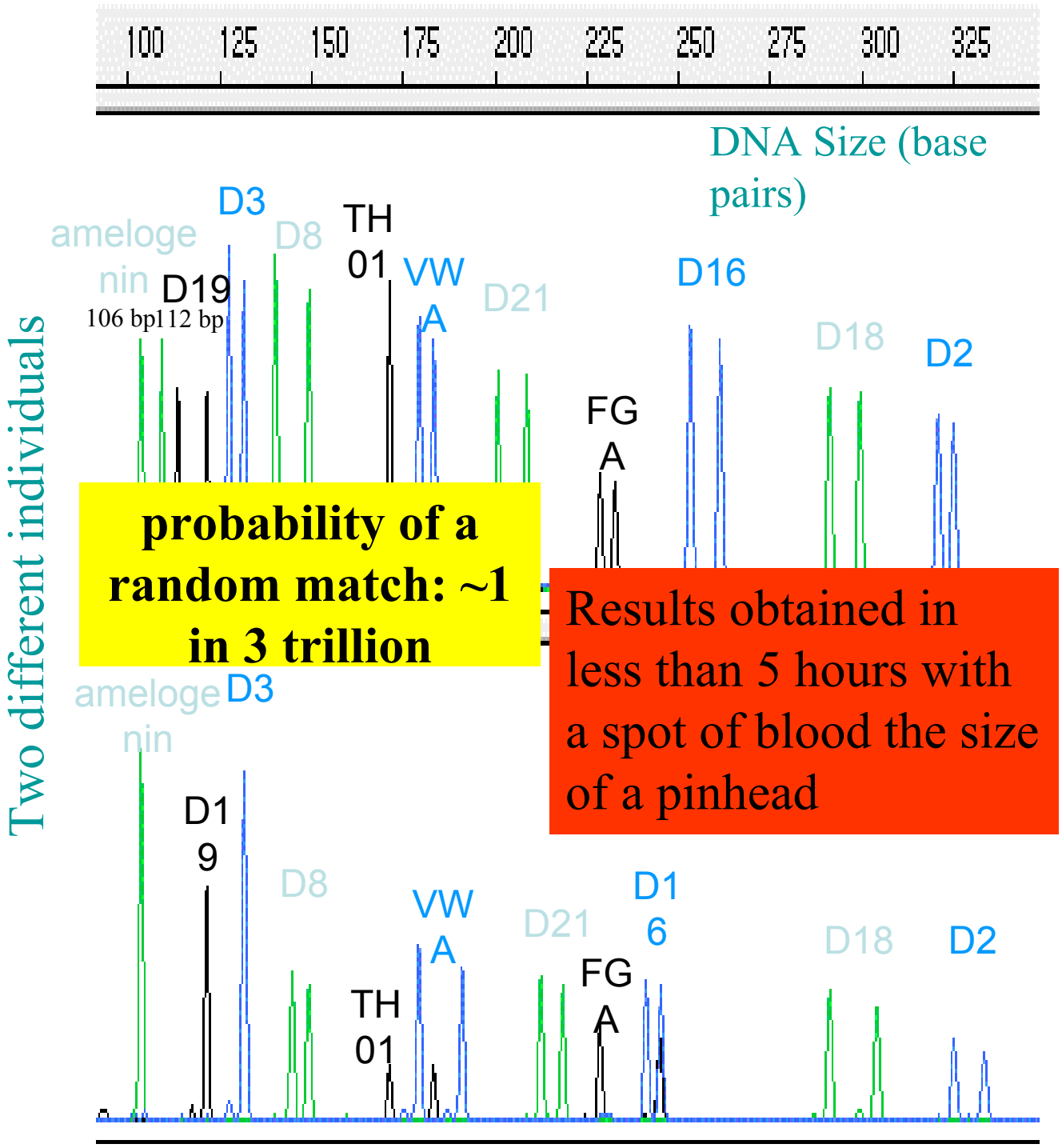
## Human Identity Testing with Multiplex STRs

Amelogenin protein is involved in tooth enamel and happens to be on sex chromosome – top: 2 peaks: x (106 bp) and y (112 bp); Bottom only 1 peak cause they have two X chromosomes.

AmpFlSTR® SGM Plus™ kit




**Simultaneous Analysis of 10 STRs and Gender ID**

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# FBI's CODIS DNA Database

## Combined DNA Index System -

-all 50 states can upload their convicted felony and seq. of unsolved cases…. In Florida to convicted felon.

- Used for linking serial crimes and unsolved cases with repeat offenders
- Launched October 1998
- Links all 50 states
- Requires >4 RFLP markers and/or 13 core STR markers
- Current backlog of >600,000 samples

Except for police errors, and sufficient racial typing,

it's a done deal

# Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

3. Related to today's subject, what would you like to know more about?

4. Any helpful comments.

Answer, and turn in at the end of class.