

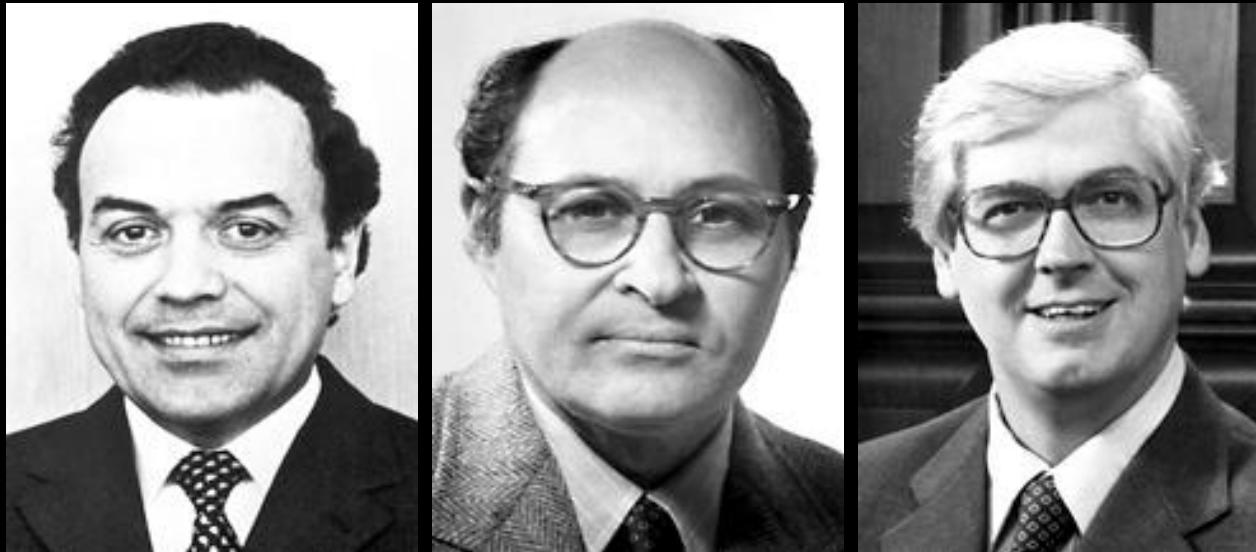
What is Genetic (Molecular) Cloning

Gene (Molecular) Cloning



The production of exact copies (clones) of a particular gene or DNA sequence using genetic engineering techniques.

The Nobel Prize in Physiology or Medicine 1978



The Nobel Prize in Physiology or Medicine 1978 was awarded jointly to **Werner Arber, Daniel Nathans and Hamilton O. Smith** '*for the discovery of restriction enzymes and their application to problems of molecular genetics*'



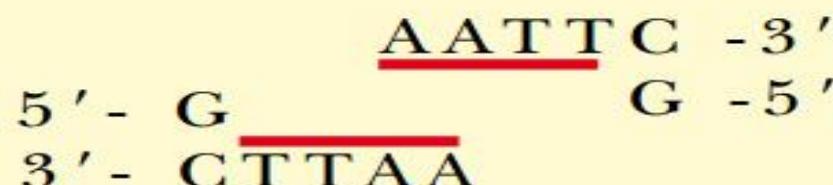
CUT BY *Hpa*1



BLUNT ENDS

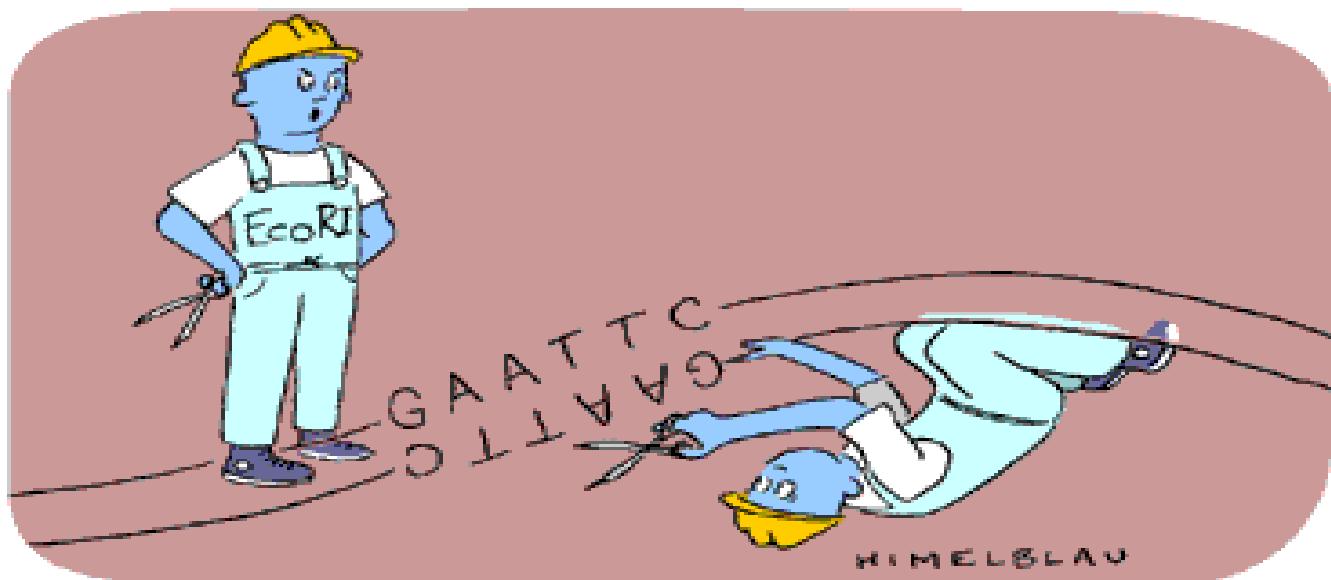


CUT BY *Eco*R1



STICKY ENDS

**Said EcoRI, "It's a curse!
The tedium couldn't be worse!
I'm stuck in a rut,
Every sequence I cut,
Is the same when it's read in reverse!"**



"Watch it, Buddy, I was here first!"

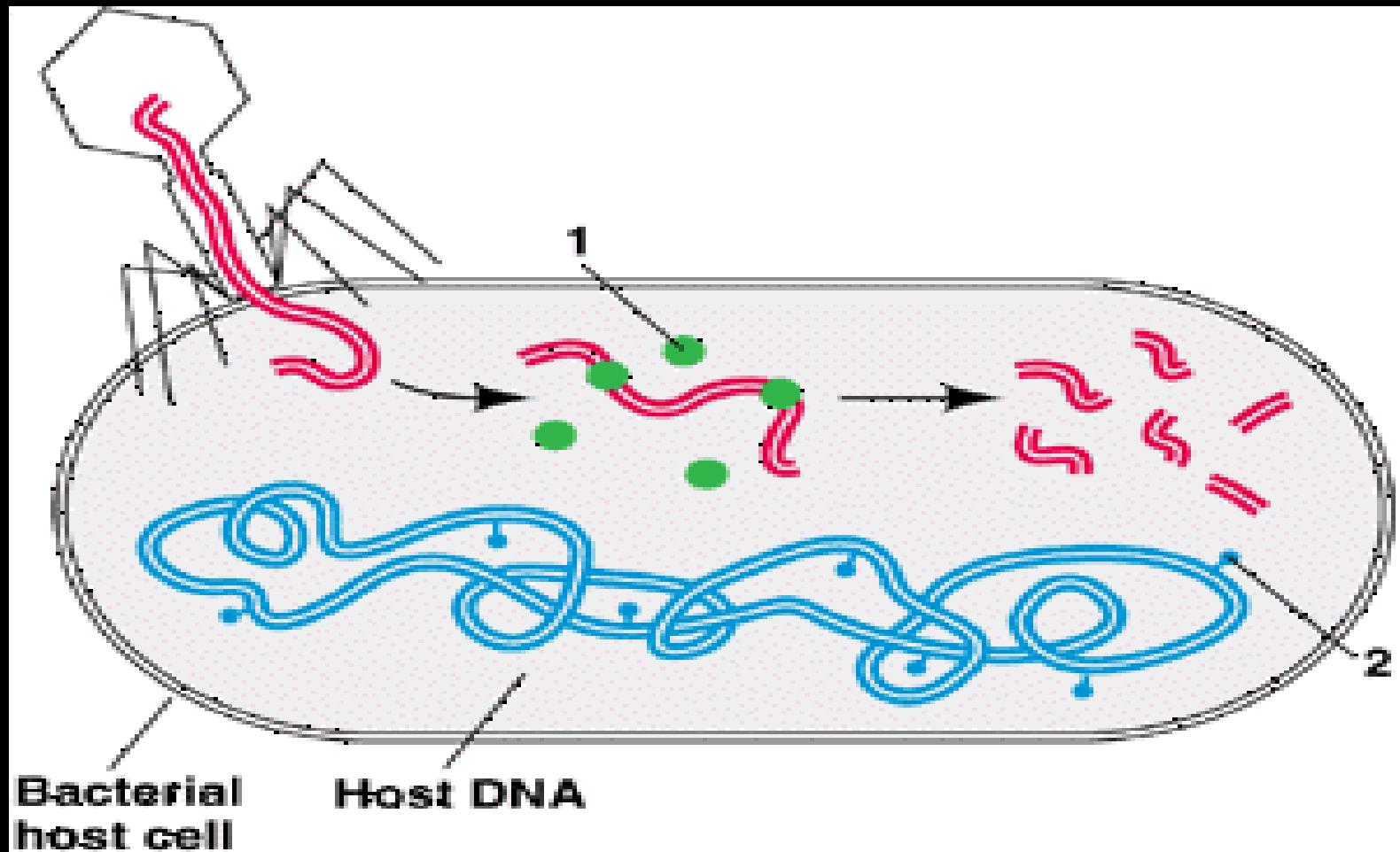
Nomenclature of restriction enzyme

Derivation of the EcoRI name		
Abbreviation	Meaning	Description
E	<i>Escherichia</i>	genus
co	<i>coli</i>	species
R	RY13	strain
I	First identified	order of identification in the bacterium

Each enzyme is named after the bacterium from which it was isolated using a naming system based on bacterial genus, species and strain.

<i>Microorganisms</i>	<i>Restriction enzymes</i>	<i>Cleavage sites</i>	<i>Cleavage products</i>	
<i>Bacillus amy-</i> <i>loliuefaciens H</i>	<i>Bam</i> HI	↓ 5-GGATCC-3 3-CCTAGG-5 ↑	5-G	GATCC-3 3-CCTAG G-5
<i>B. globigii</i>	<i>Bgl</i> II	↓ 5-AGATCT-3 3-TCTAGA-5 ↑	5-A	GATCT-3 3-TCTAG A-5
<i>Escherchia coli RY13</i>	<i>Eco</i> RI	↓ 5-GAATTTC-3 3-CTTAAG-5 ↑	5-G	AATTC-3 3-CTTAA G-5
<i>Haemophilus influenzae Rd</i>	<i>Hin</i> dIII	↓ 5-AAGCTT-3 3-TTCGAA-5 ↑	5-A	AGCTT-3 3 -TTCGA A-5
<i>H. parainfluenzae</i>	<i>Hpa</i> I	↓ 5-GTTAAC-3 3-CAATTG-5 ↑ ↓	5-GTT 3-CAA	AAC-3 TTG-5
<i>Klebsiella pneumoniae</i> OK 8	<i>Kpn</i> I	↓ 5-GGTACC-5 3-CCATGG-3 ↑	5-GGTAC 3-C	C-3 CATGG-5
<i>Streptomyces albus</i> G	<i>Sal</i> I	↓ 5-GTCGAC-3 3-CAGCTG-5 ↑	5-G 3-CAGCT	TCGAC-3 G-5
<i>Staphylococcus aureus</i> 3AI	<i>Sau</i> 3AI	↓ 5-GATC-3 3-CTAG-5 ↑	5- 3-CTAG	GATC-3 5

Why do Bacteria have Restriction Enzymes





**Why is it that the Restriction Enzymes
don't cut the bacterial DNA itself?**

**1950-60s-Around this period of time
the problem scientists were then
facing was how bacteria acquired
antibiotic resistance...**

Lederberg

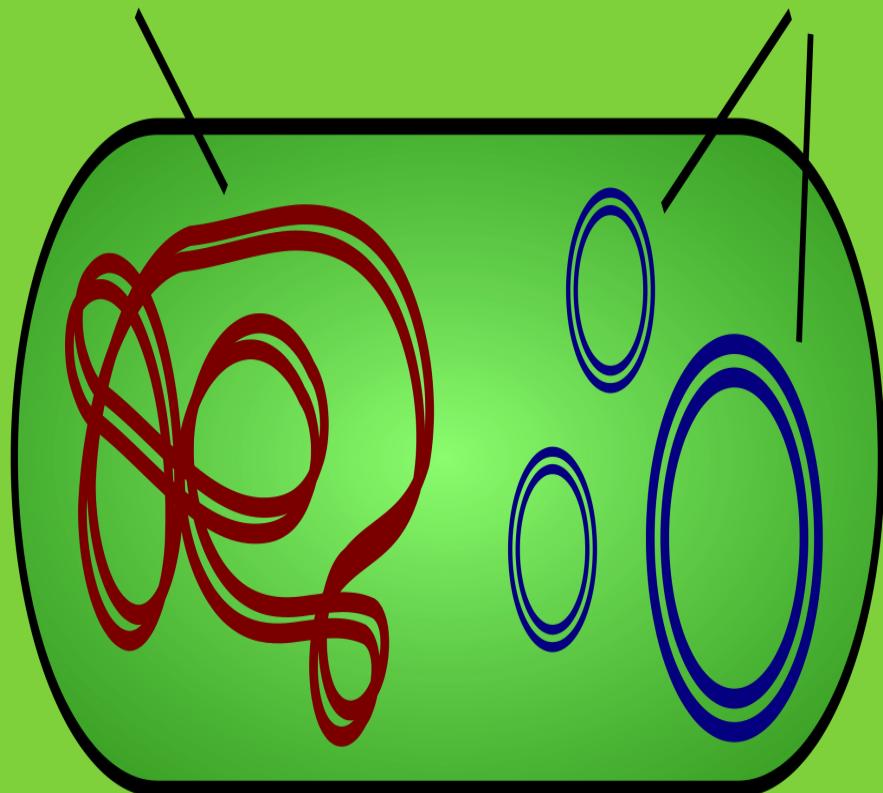


“bacterial cells differ in fundamental ways from cells in higher organisms, and this laid the foundation for genetic engineering and modern biotechnology”

Plasmids

Bacterial DNA

Plasmids



- Plasmids are naturally occurring extrachromosomal DNA molecules.
- Plasmids are circular, double-stranded DNA.



George Wells Beadle



Edward Lawrie Tatum

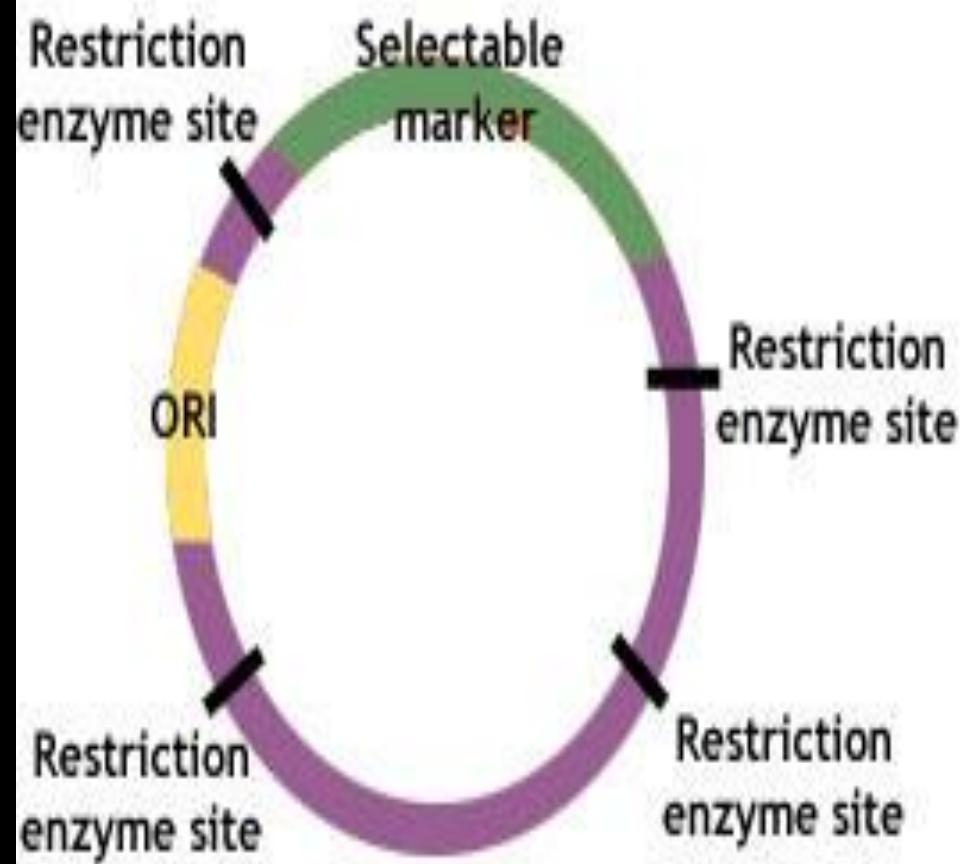
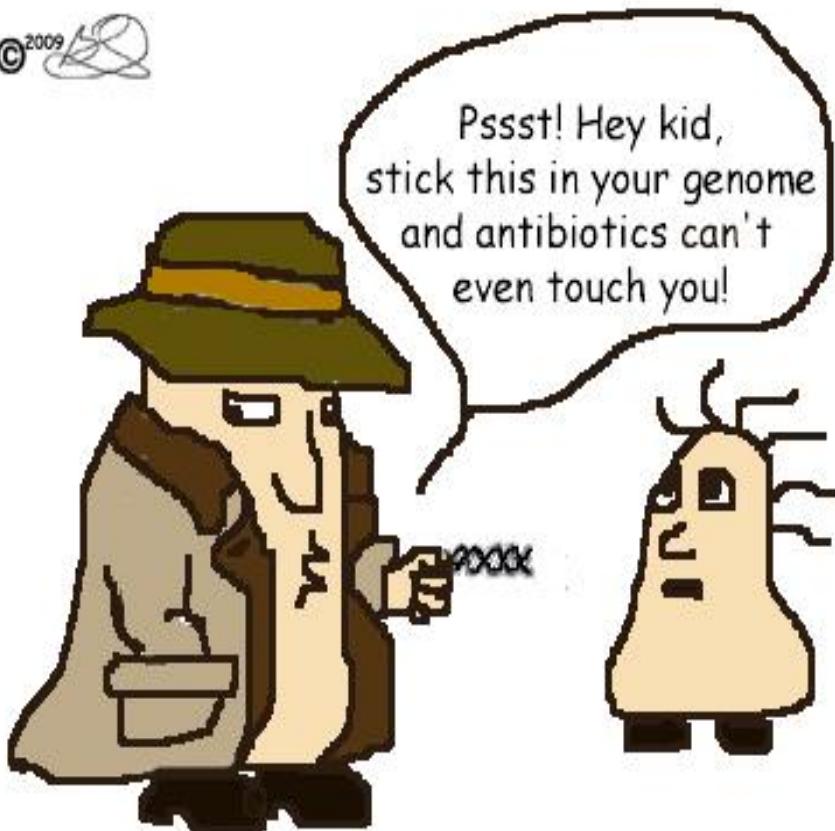


Joshua Lederberg

- The Nobel Prize in Physiology or Medicine 1958 was divided, one half jointly to George Wells Beadle and Edward Lawrie Tatum "for their discovery that **genes act by regulating definite chemical events**" and the other half to Joshua Lederberg "for his discoveries concerning **genetic recombination and the organization of the genetic material of bacteria**"(1951).

Advantage of Having a Plasmid

© 2009





Why doesn't every bacterial cell in the universe have all the possible plasmids, if the procedure is so efficient.

How the discovery of Plasmids facilitated the discovery of coning a gene?

**Plasmids can be cleaved by
restriction enzymes, leaving sticky
or blunt ends.**

The advent of recombinant DNA technology

Nobel Prize 1980: Paul Berg, Herbert W. Boyer, and Stanley N. Cohen

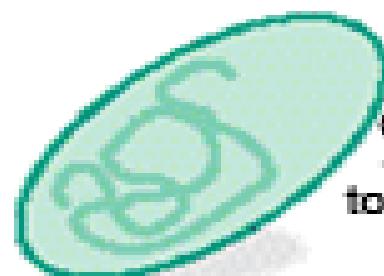


For discovering the way in which genetic material from one organism is artificially introduced into the genome of another organism and then replicated & expressed by that organism.

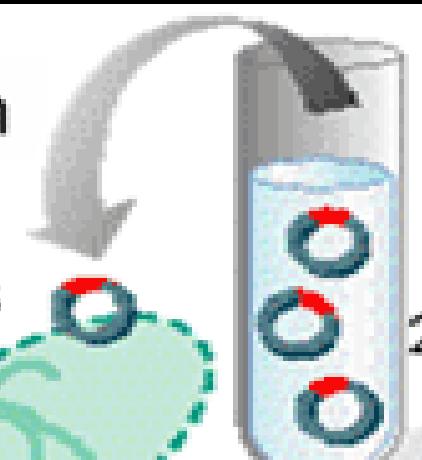
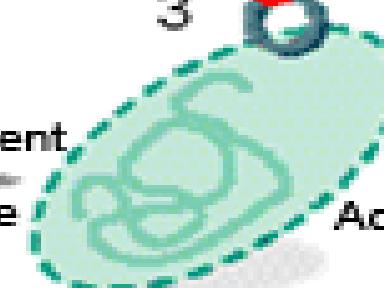
Transformation and Selection

Artificial Transformation

Antibiotic -sensitive
bacterial cell

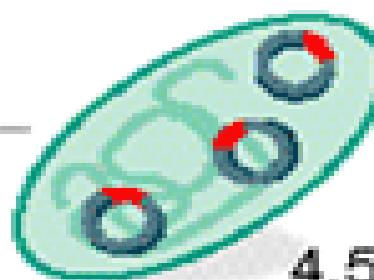


1
 $\xrightarrow{\text{CaCl}_2 \text{ treatment}}$
to permeabilize
cell walls

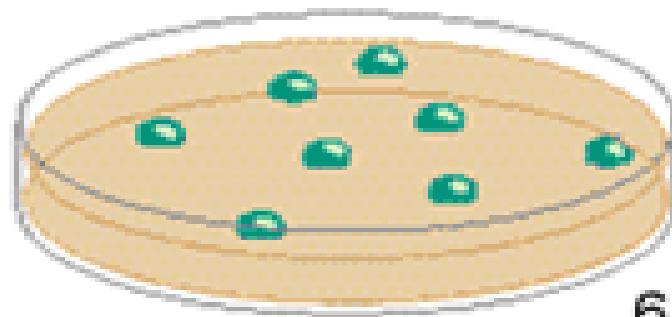


2

Add Plasmid DNA



4,5



6

Selection on bacterial growth medium
containing appropriate antibiotic

"Transformed" bacterial cell



**When would you like to clone a
gene?**

**Did you know that intelligence genes are
inherited from mother?**





Eli Lilly & Company signed a joint-venture agreement with Genentech to develop the production process for Humulin (1982)– the FIRST biotechnology product to be in market

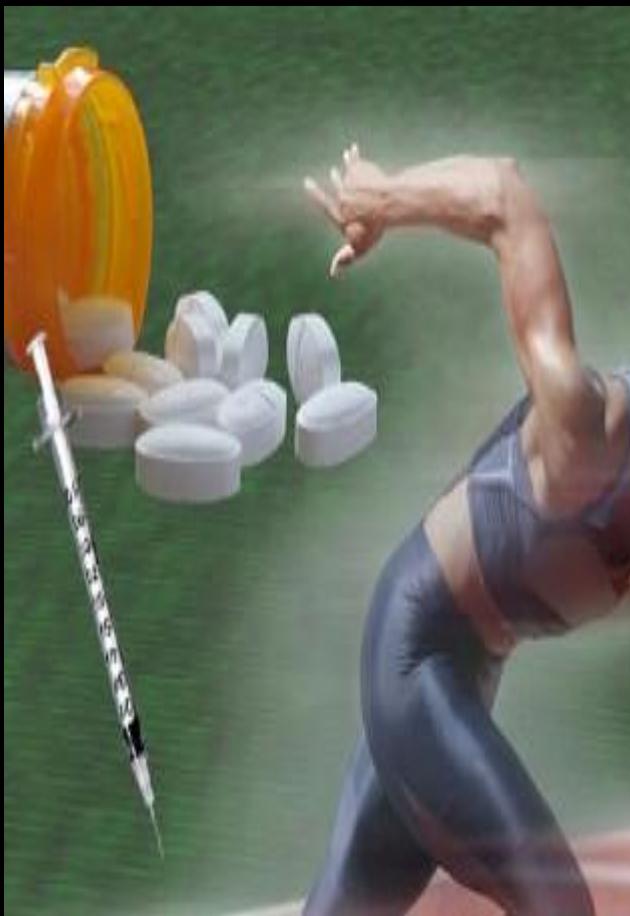
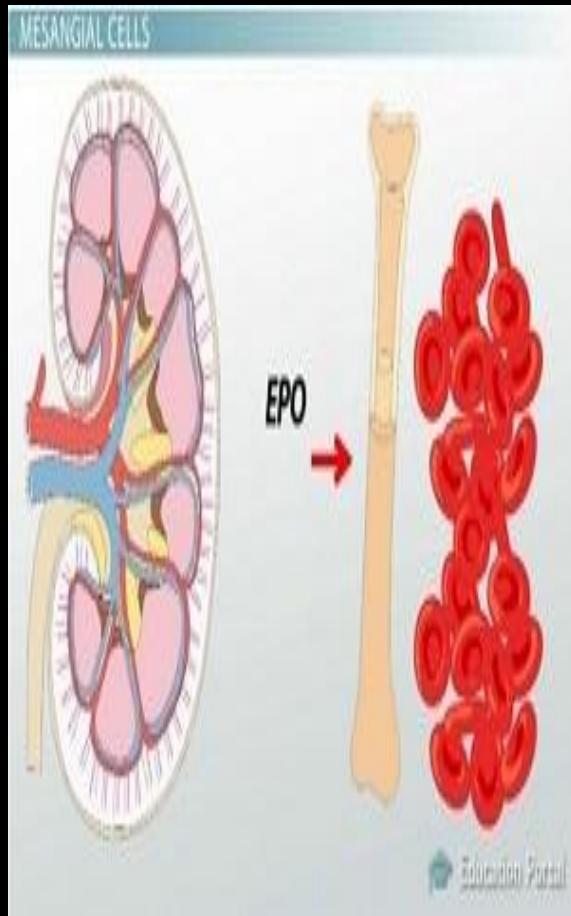
Genetically engineered HGH (1985)



**Identify this famous personality who had
HGH Deficiency**

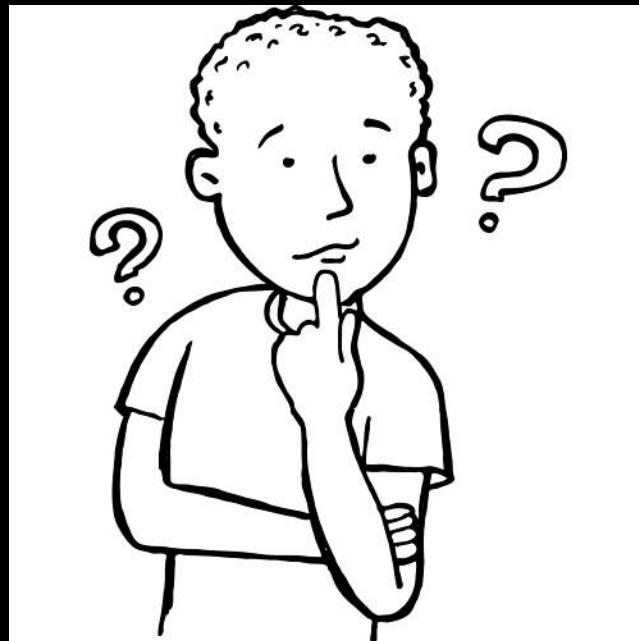


Production of Genetically Engineered Erythropoietin (2000)

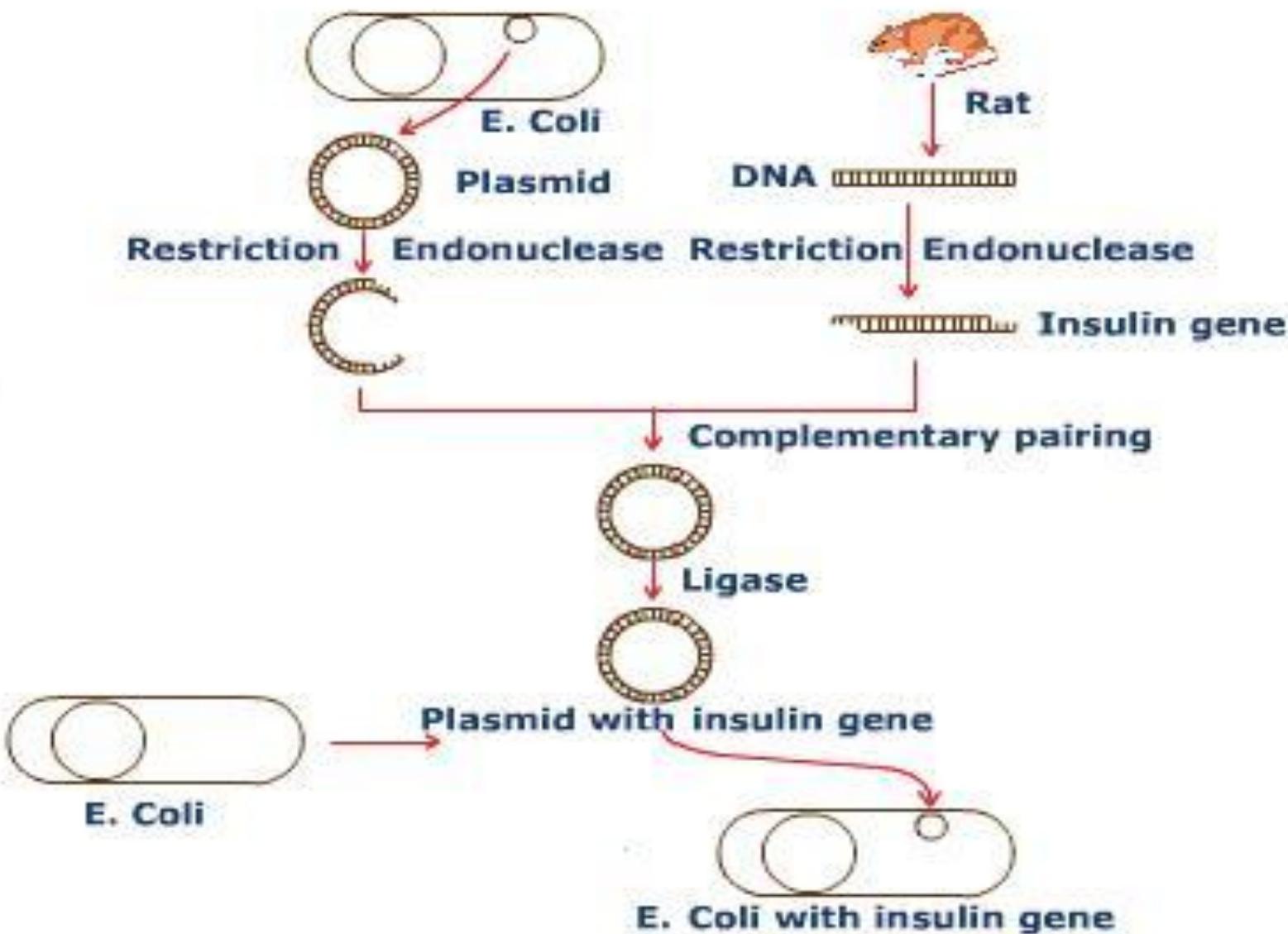


Misused by Athletes

Thought Question



An enthusiastic researcher cloned the whole rat “insulin” gene in a plasmid and transformed it to *E. coli*, but he didn’t get the desired protein product. Why?



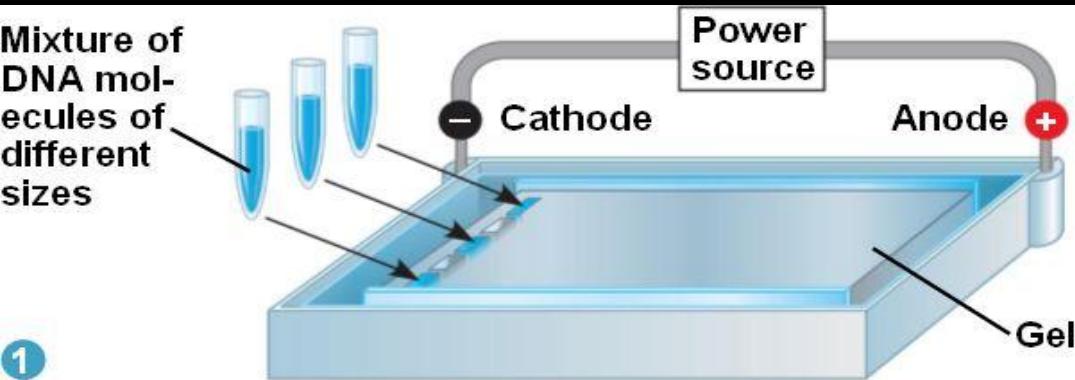
Topics Covered in Applications of Biotechnology

1. Reproductive and Therapeutic Cloning
2. Stem Cells
3. iPSC
4. Restriction enzymes
5. Gene Cloning
6. Gel electrophoresis
7. GMO
8. PCR

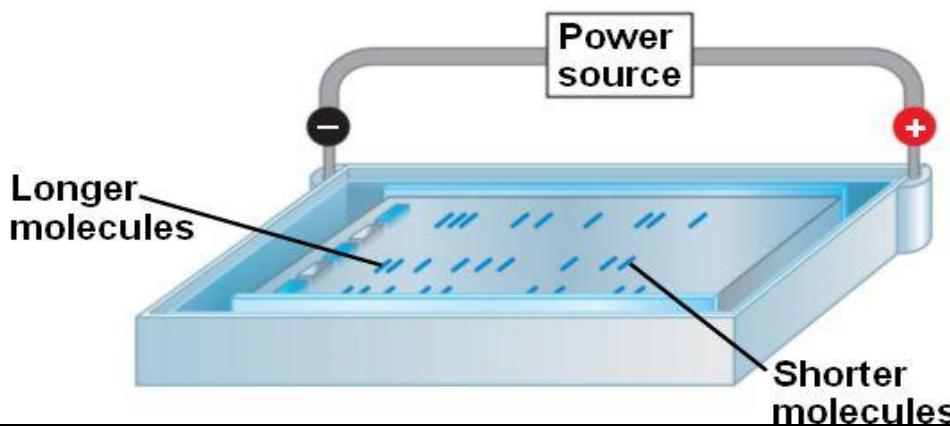
Thought Questions

1. Would you start with DNA for cloning an Eukaryotic gene?
2. What is reverse transcriptase. Why it is so called?
3. Why cant I clone in the bacterial genomic DNA itself, why do I need a plasmid to clone?
4. By cloning we are getting a desired protein, then why call it “gene cloning”?
5. How many cuts are required in a plasmid and human DNA to clone a gene?
6. How do I know that the bacteria have the plasmid with the cloned gene of interest?
7. How do I know that the plasmid didn’t anneal without the insert in it?

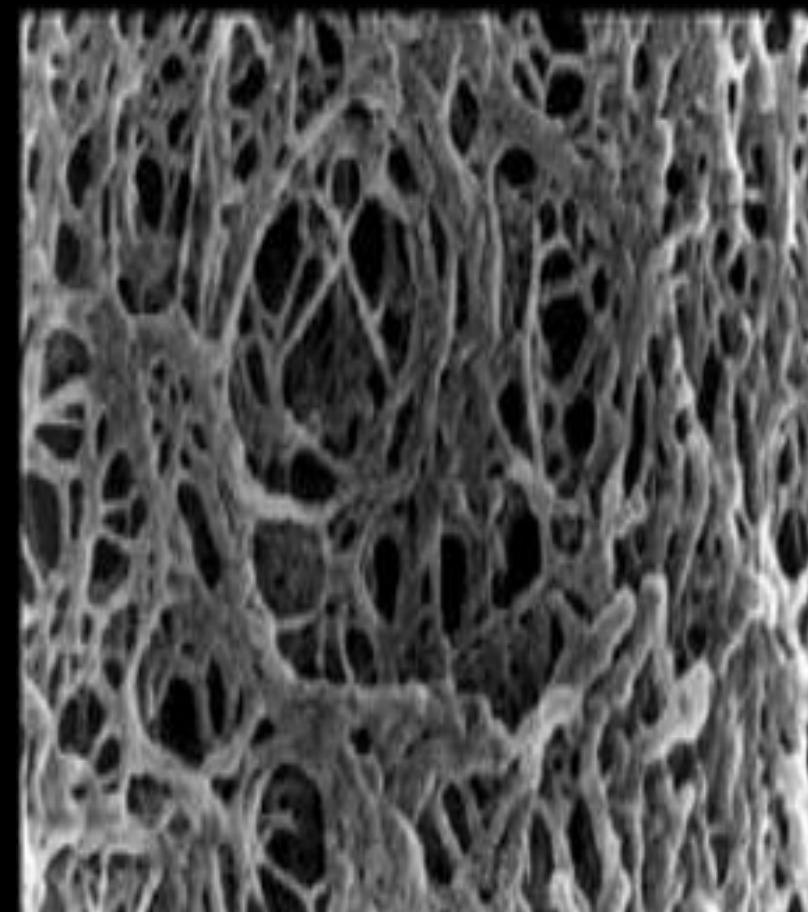
Gel Electrophoresis



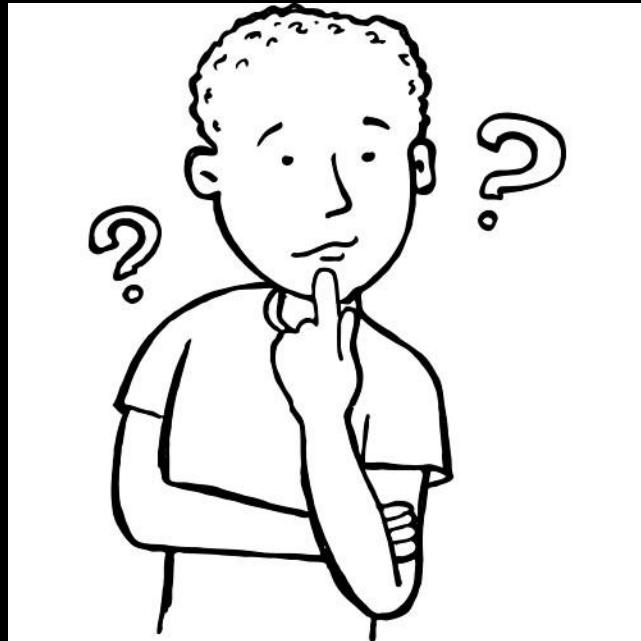
1



2

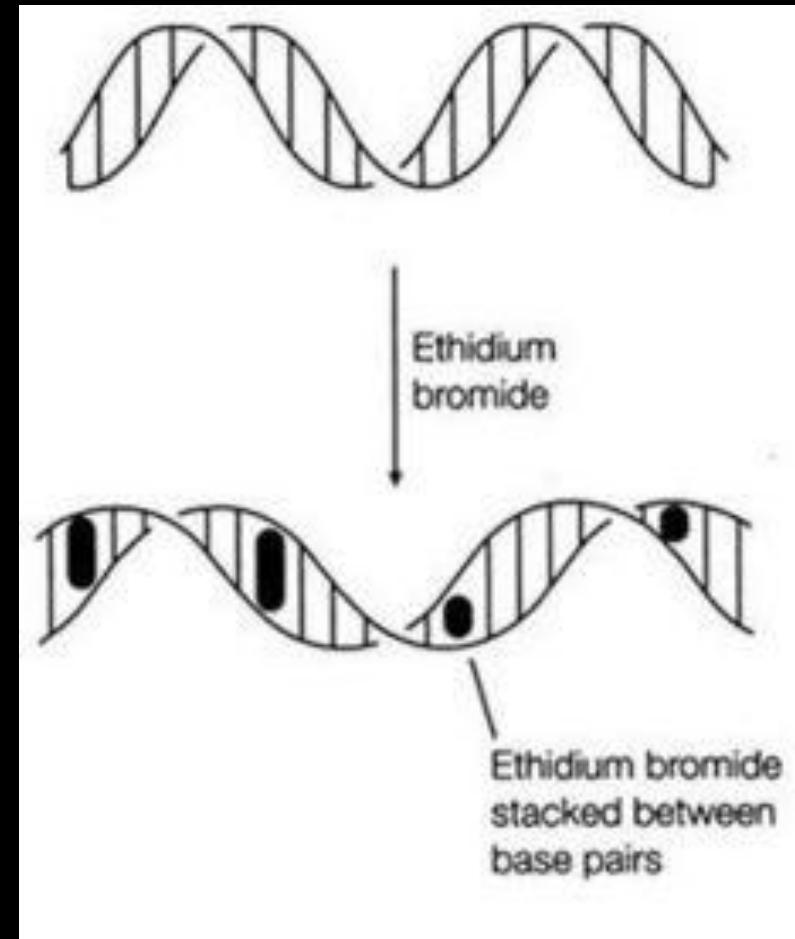
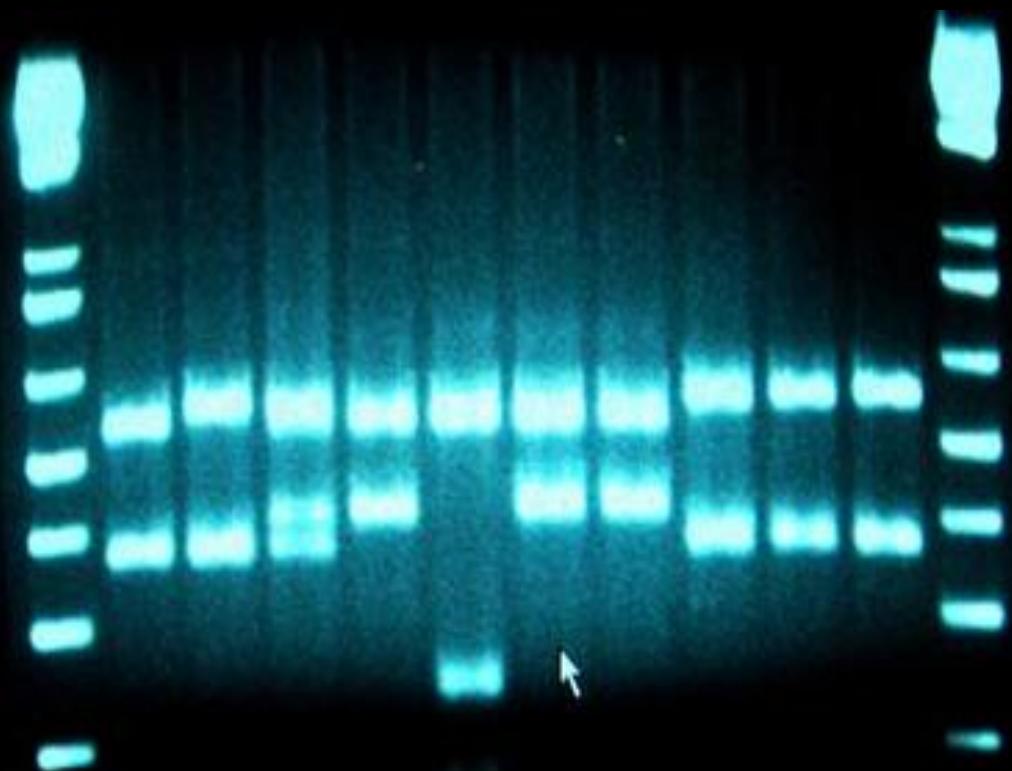


Samples from the restriction enzyme digests are introduced into the gel. Electric current is applied causing the fragments to migrate through the gel.



Can DNA be visualized through naked eye in a gel?

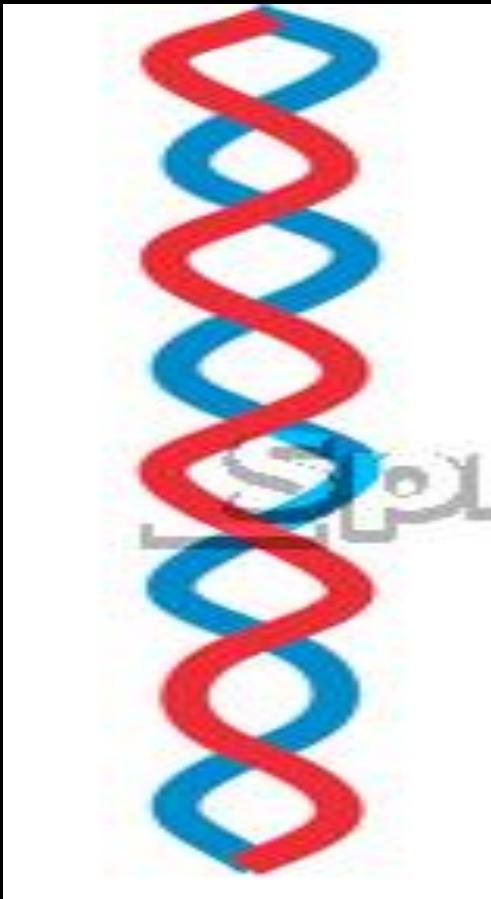
Visualizing DNA



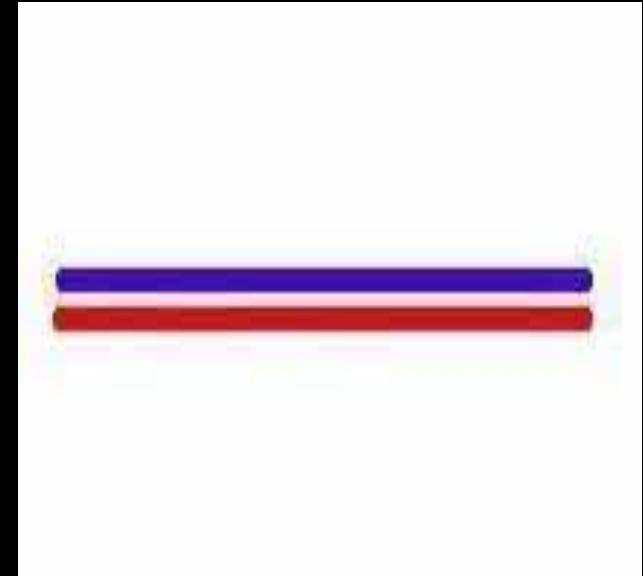
Gel is stained with a dye to allow the fragments to be visualized.

Which DNA would Migrate Faster

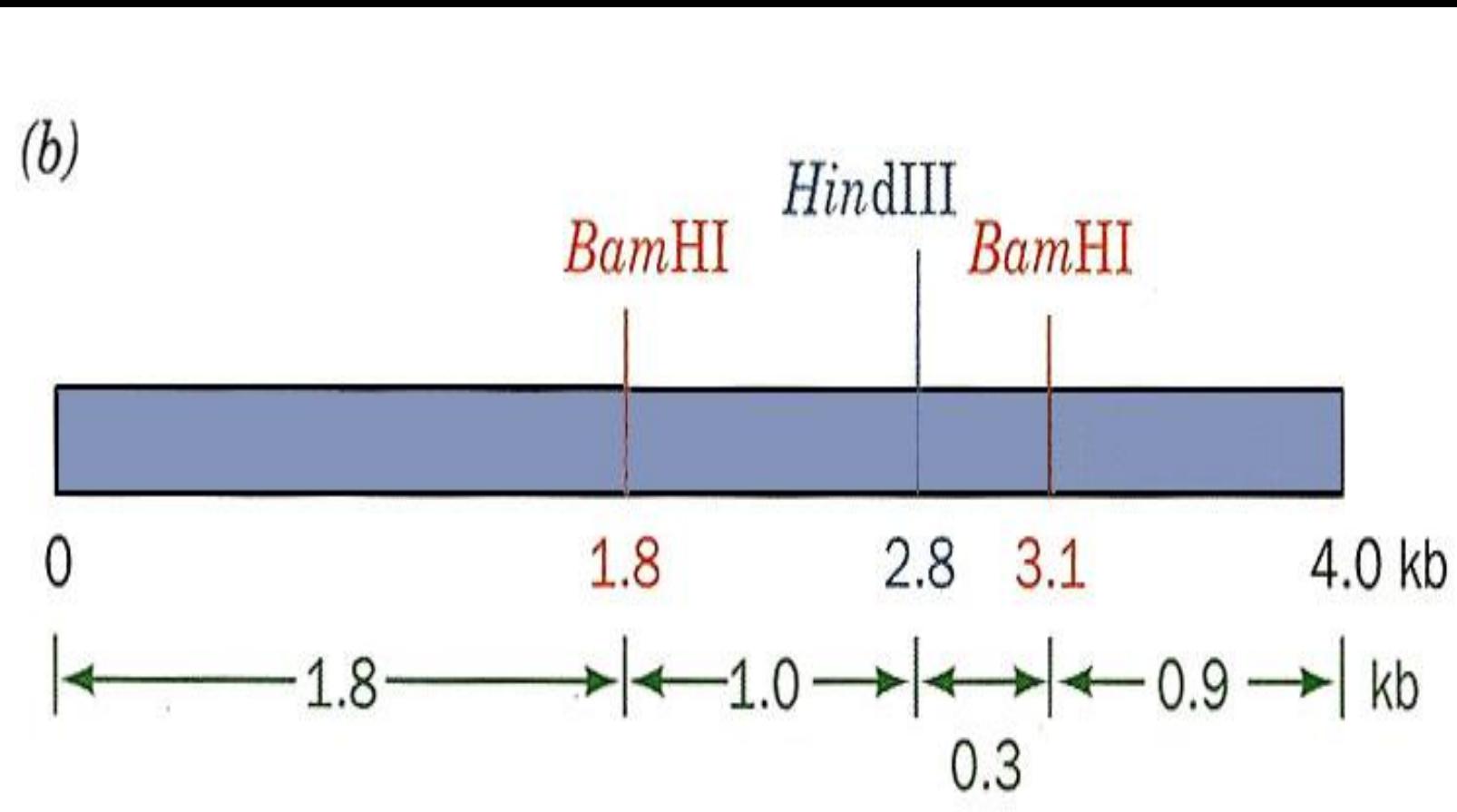
Super-coiled



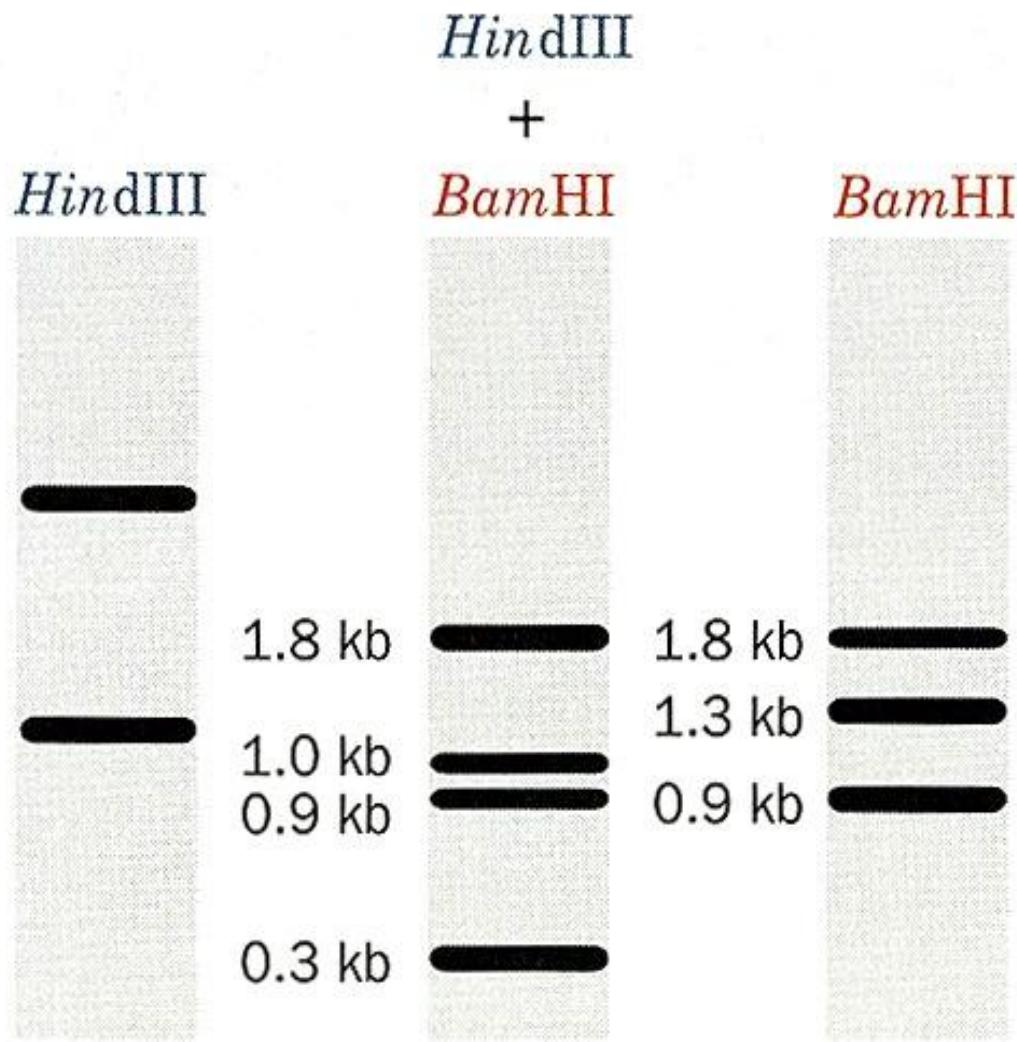
Linear



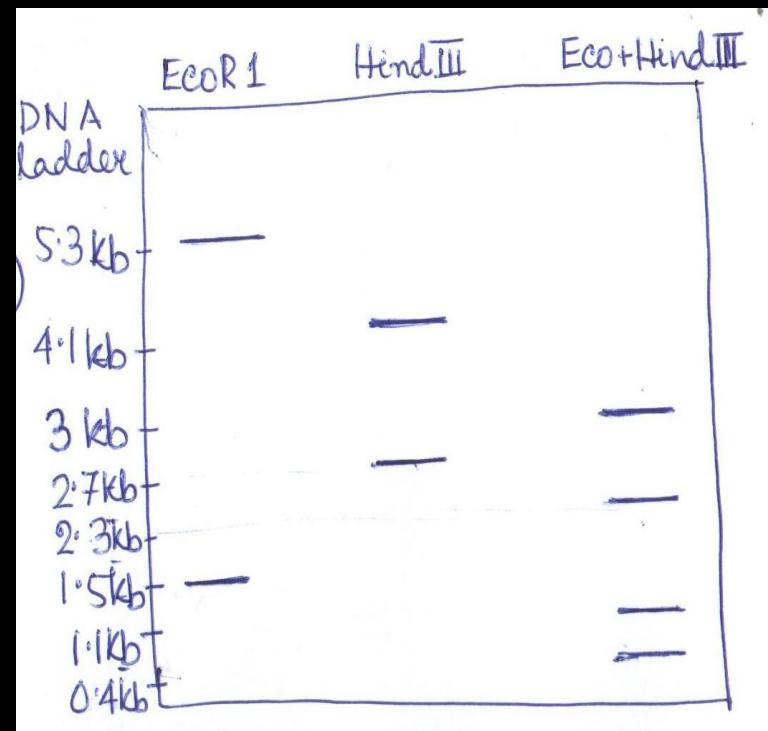
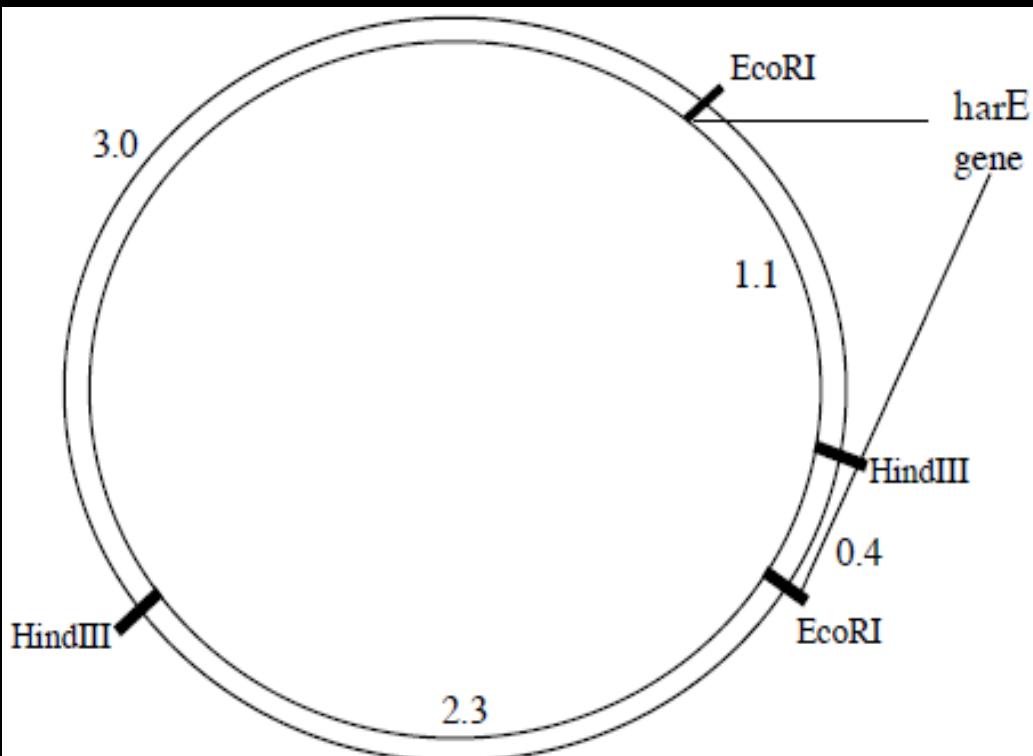
Find out the fragment sizes generated if the following DNA is cut with BamH1 / HindIII / BamH1+HindIII



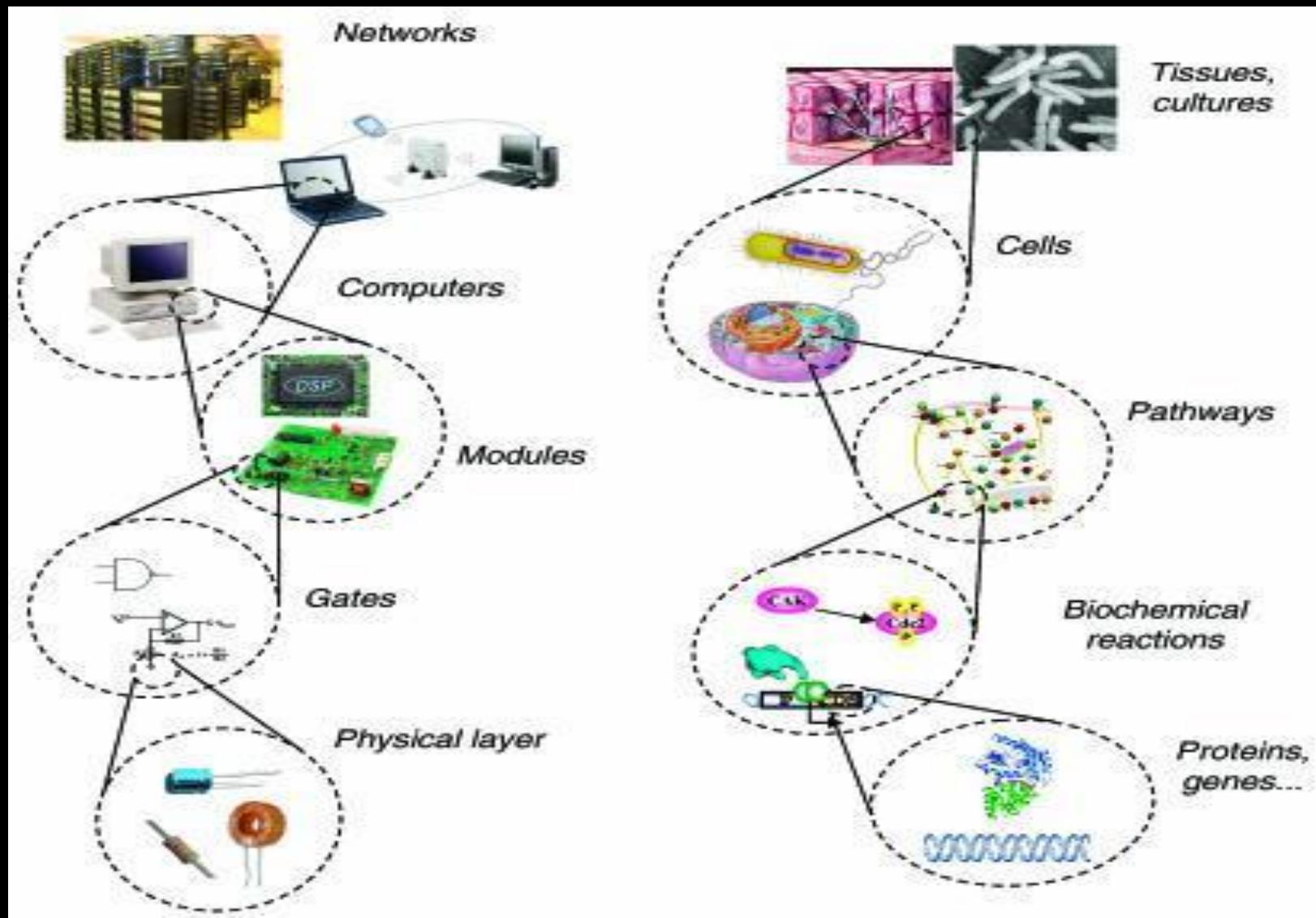
(a)



Given below is the map of the plasmid with the CHIMP-Hair gene inserted in the plasmid. The numbers denote size in kb (kilobases). Draw an agarose gel, in which the **first lane** shall have the plasmid digested with only *EcoR1 enzyme*, the **second lane** shall have the plasmid digested with only *HindIII* and **third with** *EcoR1* and *HindIII*. Draw the approximate position and mention the size of the digested DNA bands on the electrophoreses gel



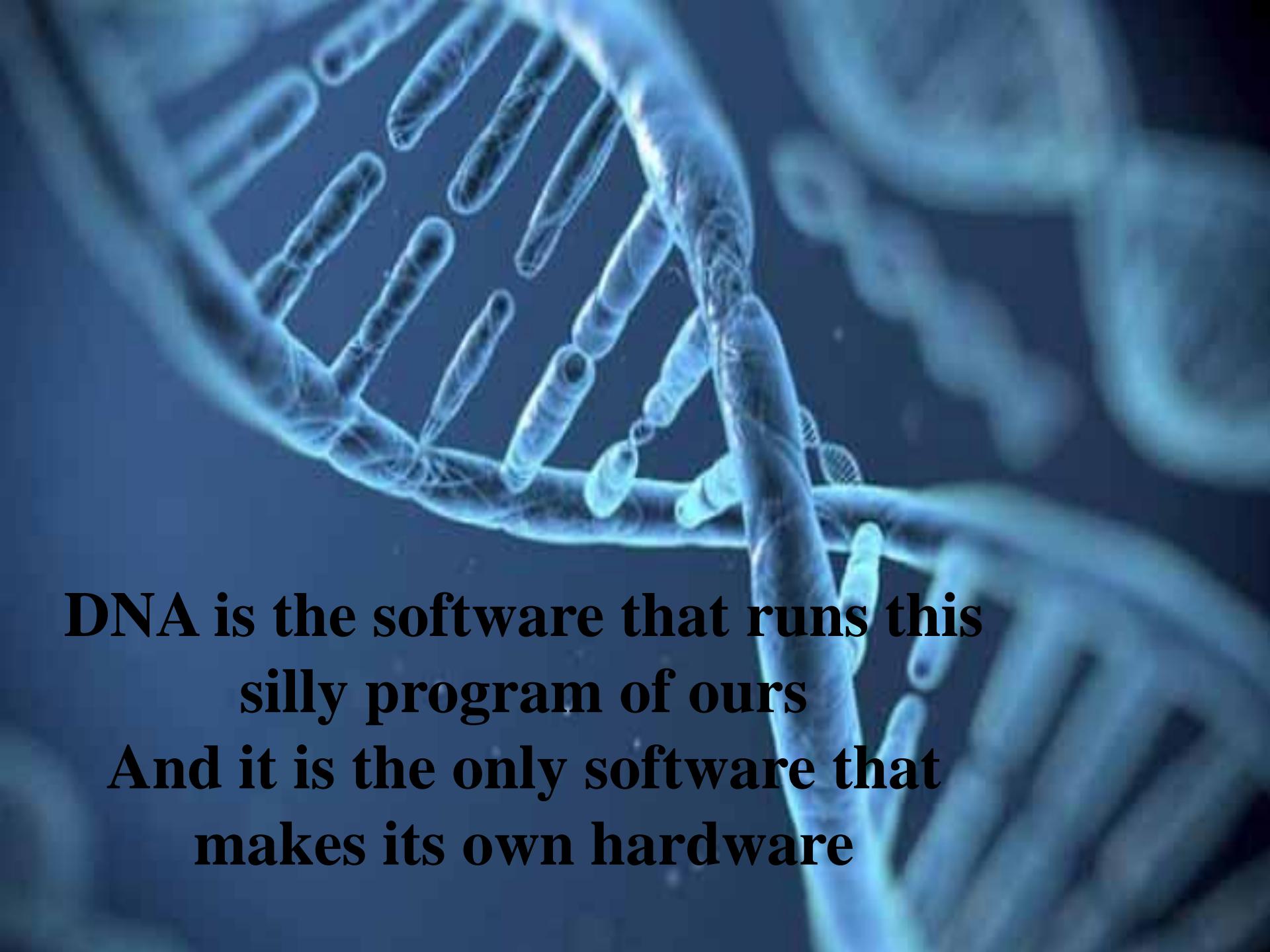
The architectures of computing networks have analogues in biology



Your cells are just computers, the
only thing is that they are way
sophisticated....

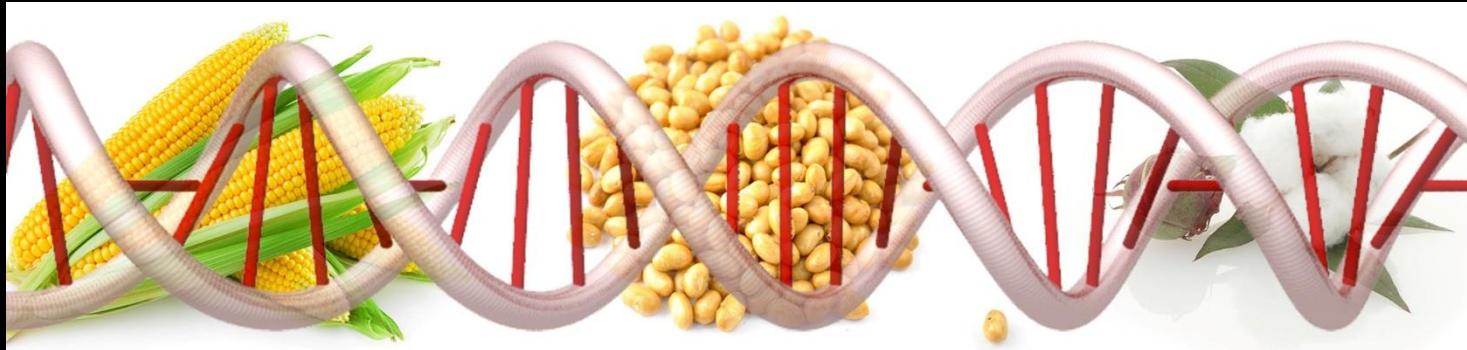


They can self manufacture

A glowing blue DNA double helix is centered against a dark, textured background. The DNA structure is composed of two interlocking spiral chains, with bright highlights along the ridges of the helix. The overall effect is a glowing, organic light source.

**DNA is the software that runs this
silly program of ours
And it is the only software that
makes its own hardware**

Science Fiction today has become our reality....genetic engineering has truly changed our life and the way we live.....



BEFORE



AFTER



DNA technology has fast replaced traditional breeding as we are more into Genetically Modified Organisms (GMOs).

Some Genetically Engineered Foods

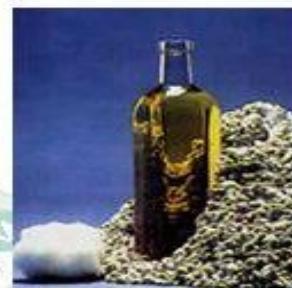
Top 10 genetically modified foods



Corn



Soy



Cottonseed



Papaya



Rice



Rapeseed
(Canola)



Potatoes



Tomatoes

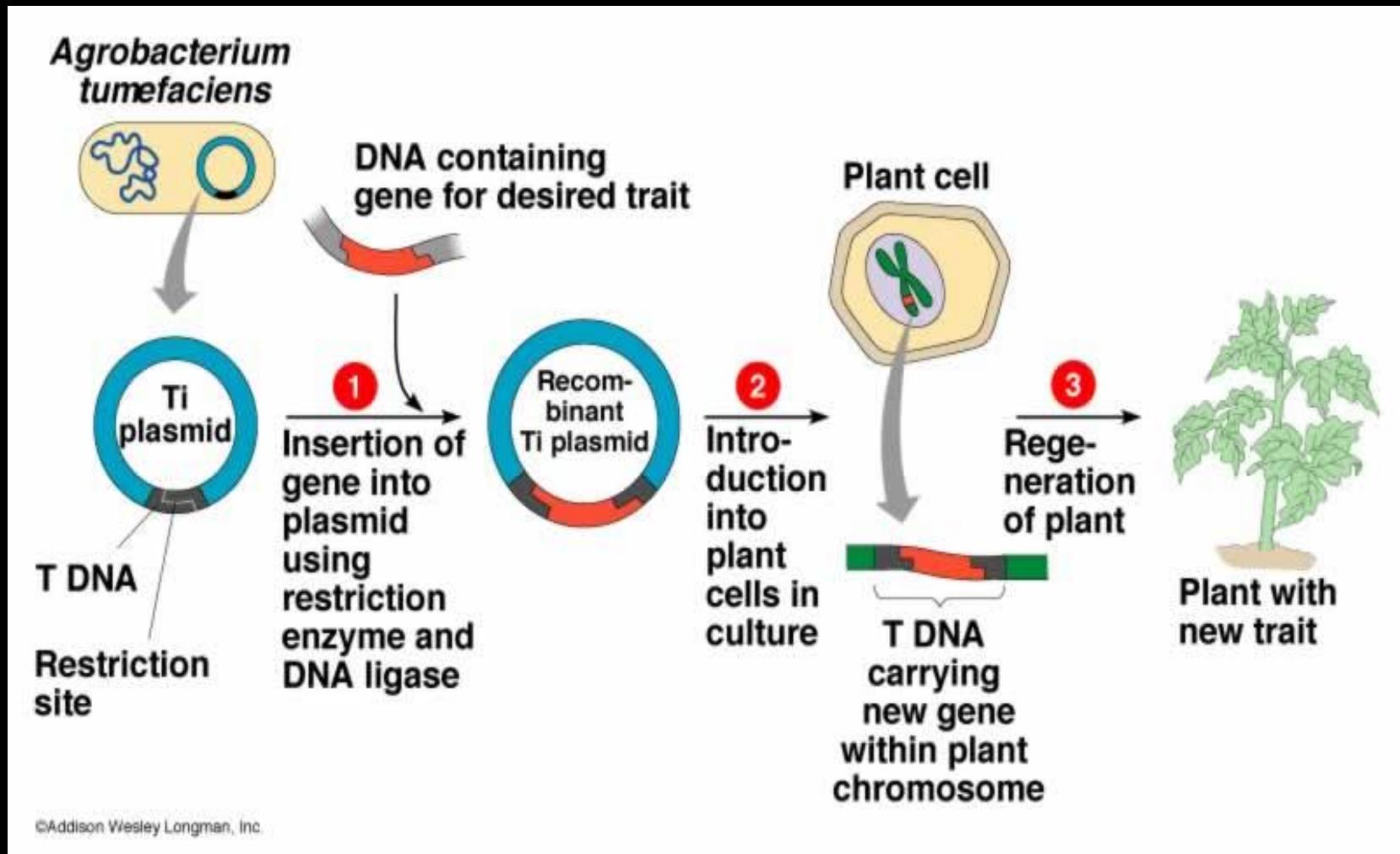


Dairy products



Peas

How to make Genetically Modified Crops



GMOs – Friend or Foe?





*Everyone has
a right to know
what's in
their food.*

Trouble in Paradise: Food for Thought



Luckily currently no genetically modified animals have been approved for human consumption

Rising suicide rate for Indian farmers blamed on GMO seeds

Published time: 22 Nov, 2014 18:42

[Get short URL](#)



Reuters/Ajay Verma / Reuters



Monsanto, which has just paid out \$2.4 million to US farmers, settling one of many lawsuits it's been involved in worldwide, is also facing accusations that its seeds are to blame for a spike in suicides by India farmers.

READ MORE: Monsanto to pay \$2.4mn to farmers over 2013 GMO-wheat scare

Tags

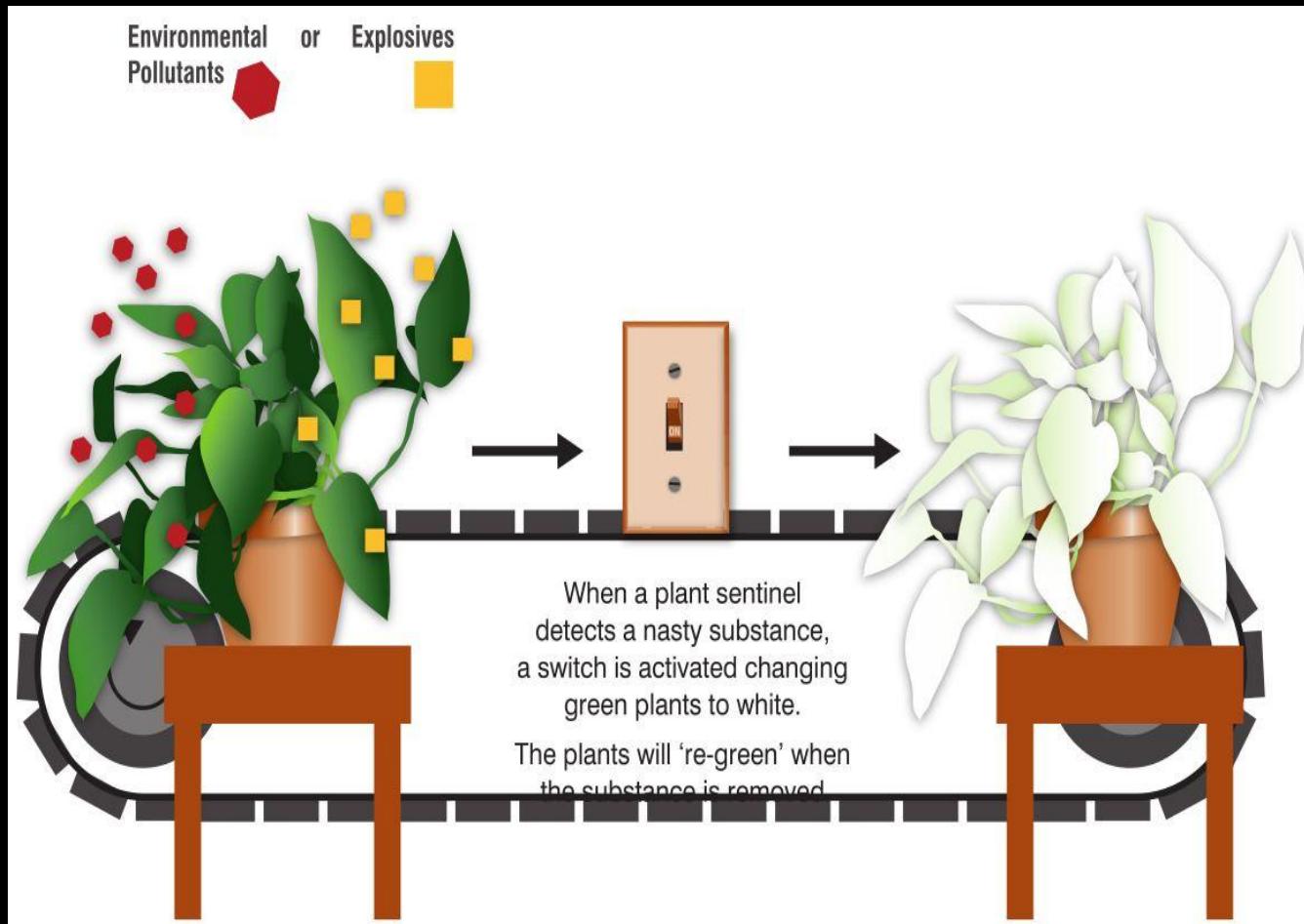
[SciTech](#), [Biology](#), [India](#), [Agriculture](#), [GMO](#)

Can you imagine using a glowing plant instead of a lamp post to light up a street?



The “stolen” glowing gene (bio-luminescence gene) are from bacteria that naturally glow. Dutch designer Daan Roosegaarde (2010).

Growing Your Own Security: Bomb-Detecting Plants (2011)



June Medford, Colorado State University and
Michael Strano, Chemical Engineer at MIT

A Brave New World of Transgenic Animals



The transgenic animals are GMOs whose genome has been edited to carry genes from other species.

The Spider-goat



In 2000, Nexia Biotechnologies announced the introduction of a goat that produced spiders' web protein in its milk. This “silk milk” could be used to manufacture a web-like material called ‘Biosteel’

Ebola - Nature, Accident or Intentional

Scientists allege deadly diseases such as *Ebola* are “Bio weapons”

Ebola: Genetically Modified Organism developed in US Biowarfare Laboratories in Africa.

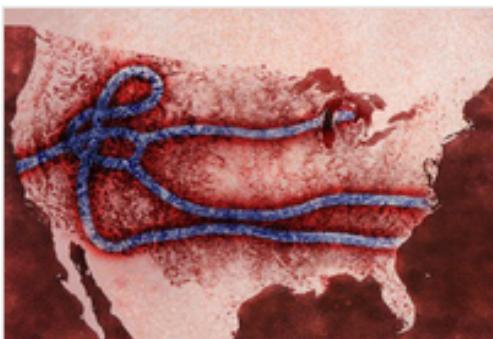
By [Dr. Paul Craig Roberts](#)

Global Research, October 21, 2014

paulcraigroberts.org

Region: sub-Saharan Africa

Theme: Militarization and WMD, Science and Medicine



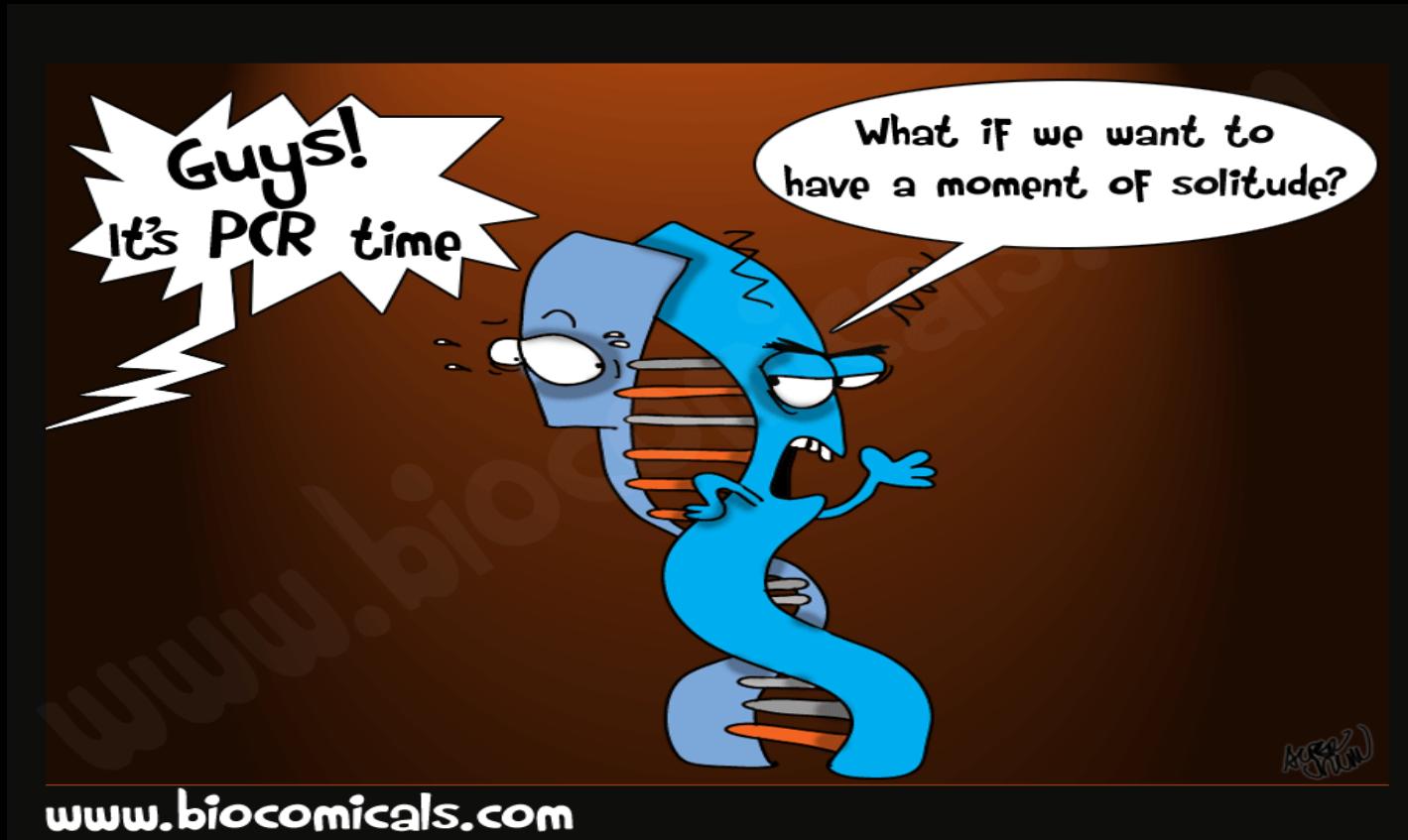
As I read this notice from [ClinicalTrials.gov](#), a service of the US National Institutes of Health, the US Government and Pharmaceutical corporations have been conducting ebola tests on humans. <http://clinicaltrials.gov/show/NCT02041715>

This is official confirmation of Dr. Boyle and Dr. Broderick's reports that the US government has conducted ebola experiments. Perhaps the vaccine was not effective, and those on whom the

Leaving a trail



Polymerase Chain Reaction



**Method for Exponential Amplification of
Nucleic Acid Fragments**

DR.KARY BANKS MULLIS

DISCOVERY OF PCR

Dr.Kary Banks Mullis received a Nobel Prize in chemistry in 1993, for his invention of the polymerase chain reaction (PCR). The process, which Kary Mullis conceptualized in 1983, is hailed as one of the monumental scientific techniques of the twentieth century.



T.V.RAO MD

2

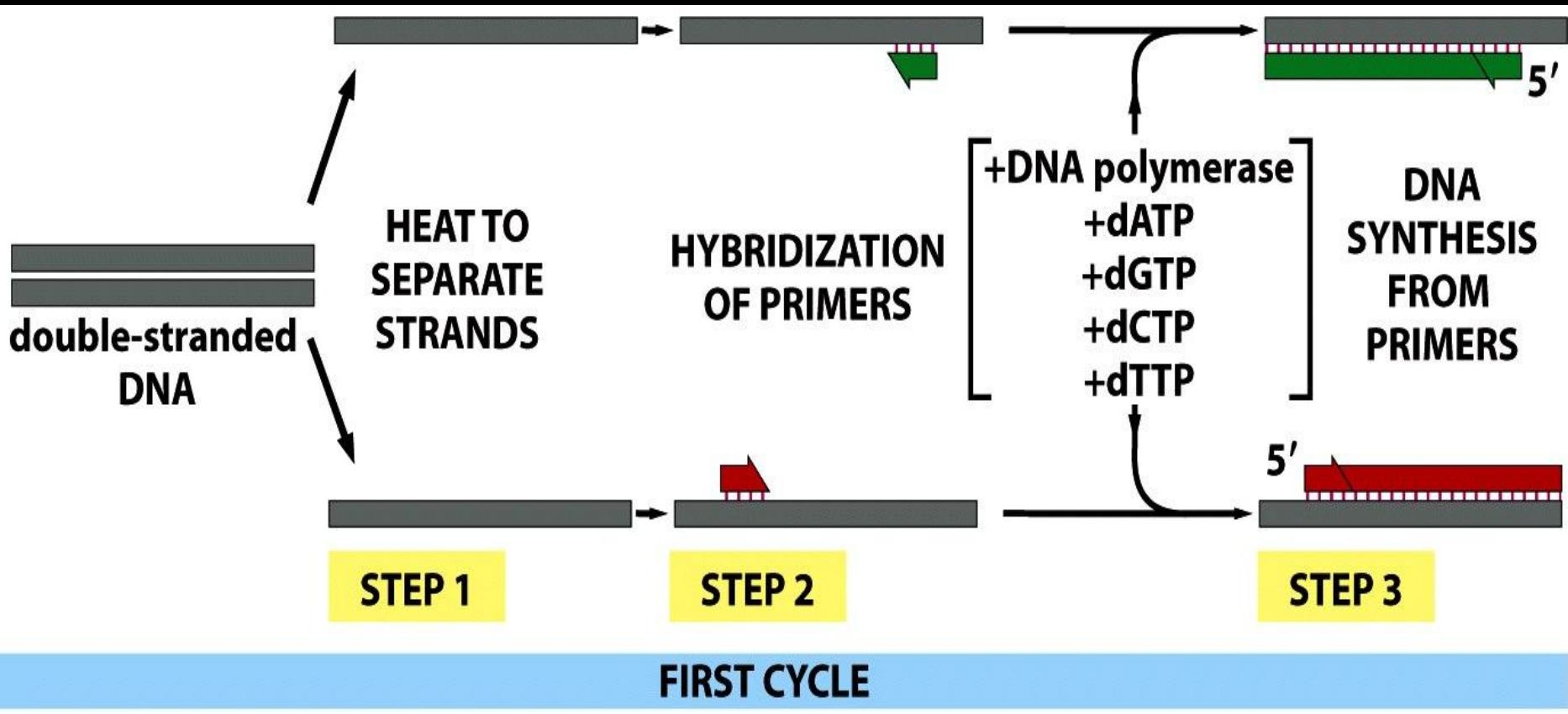
The Discovery of PCR was a Revolution

The Eureka Moment...

“ The revelation came to me one Friday night in April, 1983, as I gripped the steering wheel of my car and snaked along a moonlit mountain road into Northern California’s redwood county...” -

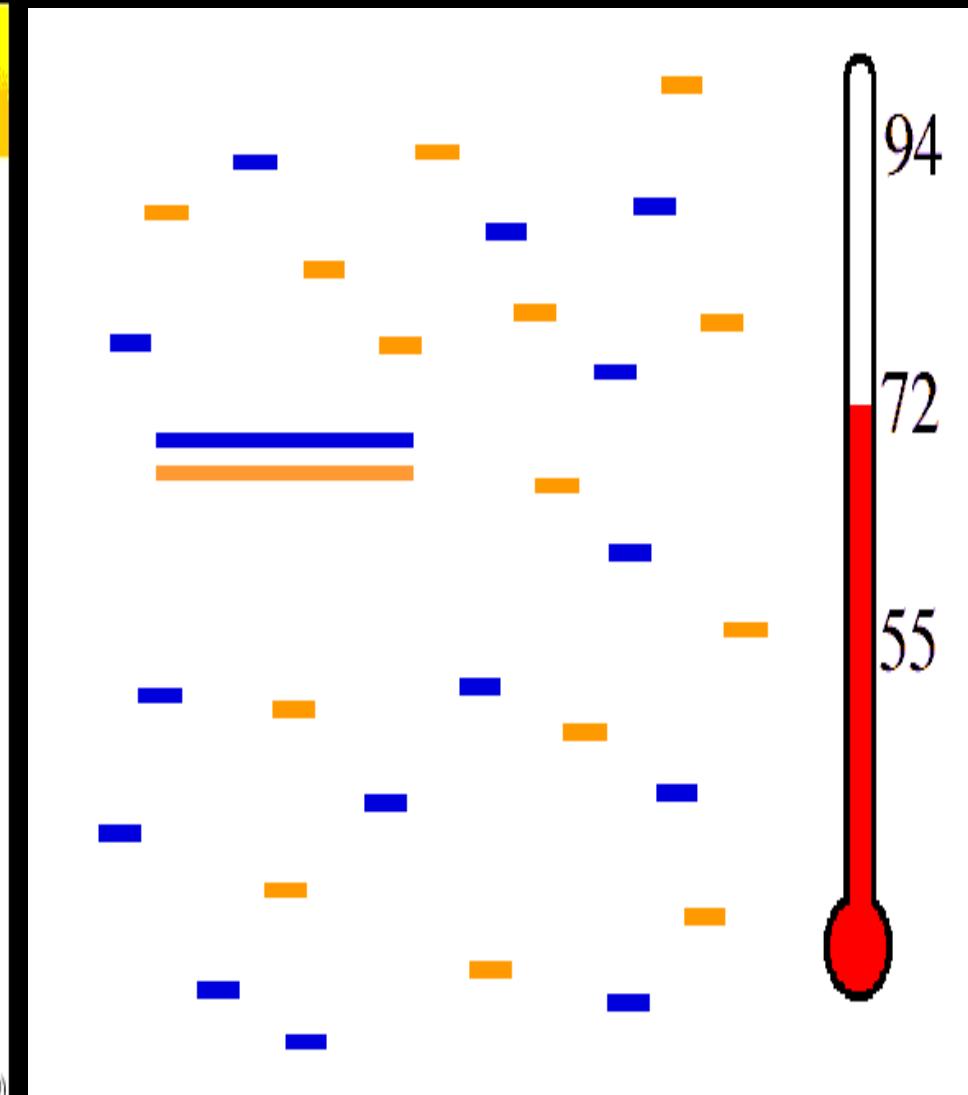
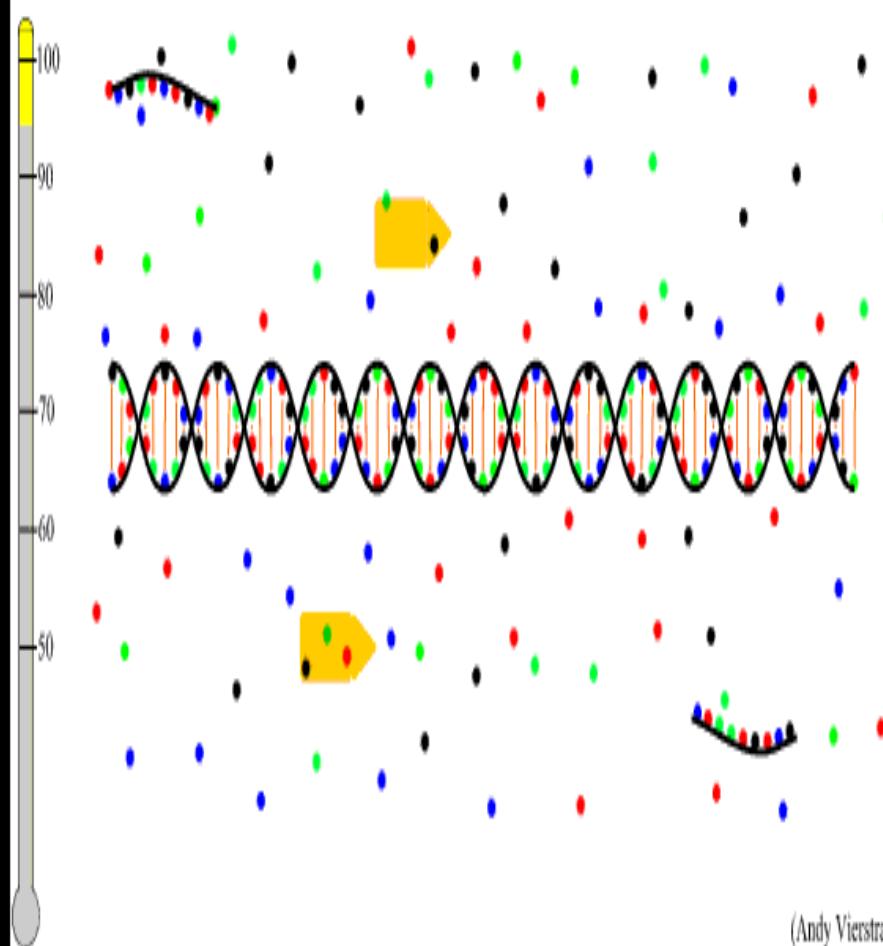
-----Kary Mullis

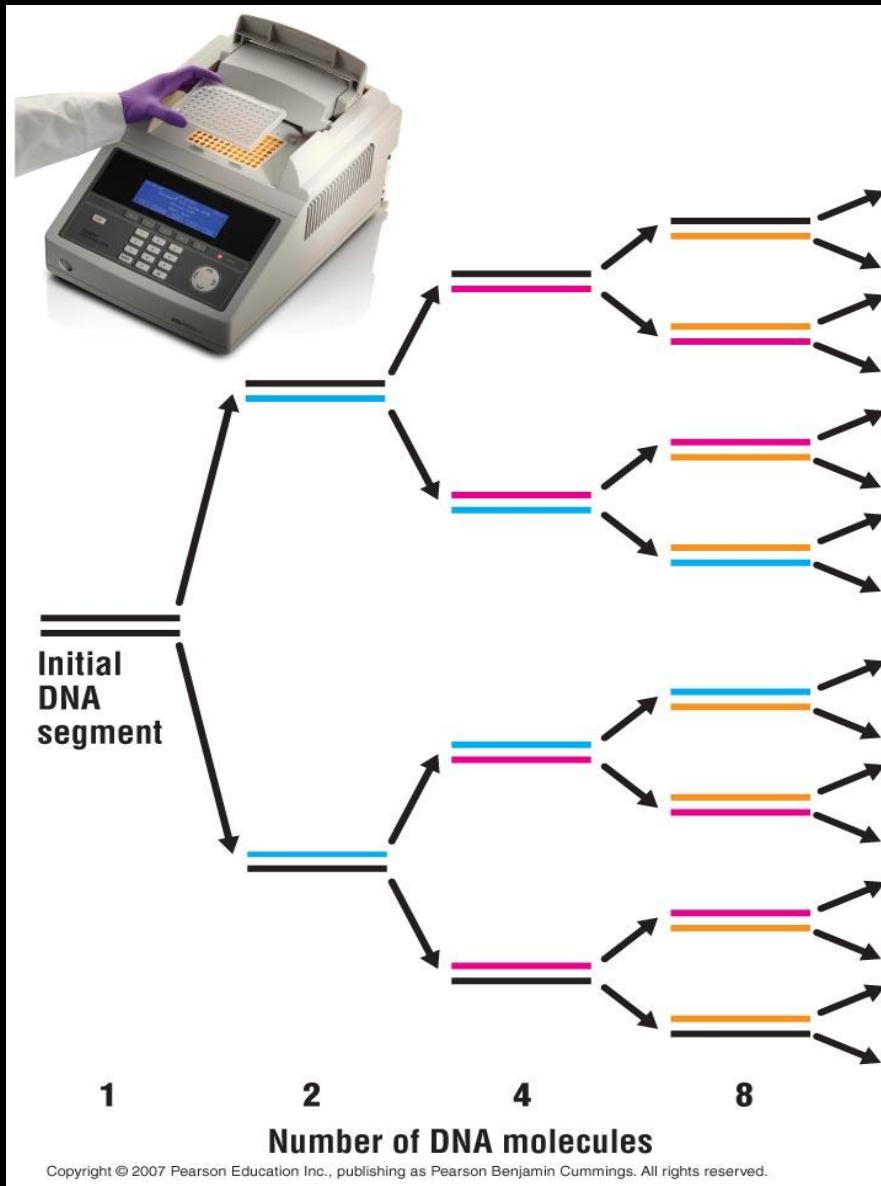
Polymerase Chain Reaction (PCR)



PCR:

Denaturation 94°C

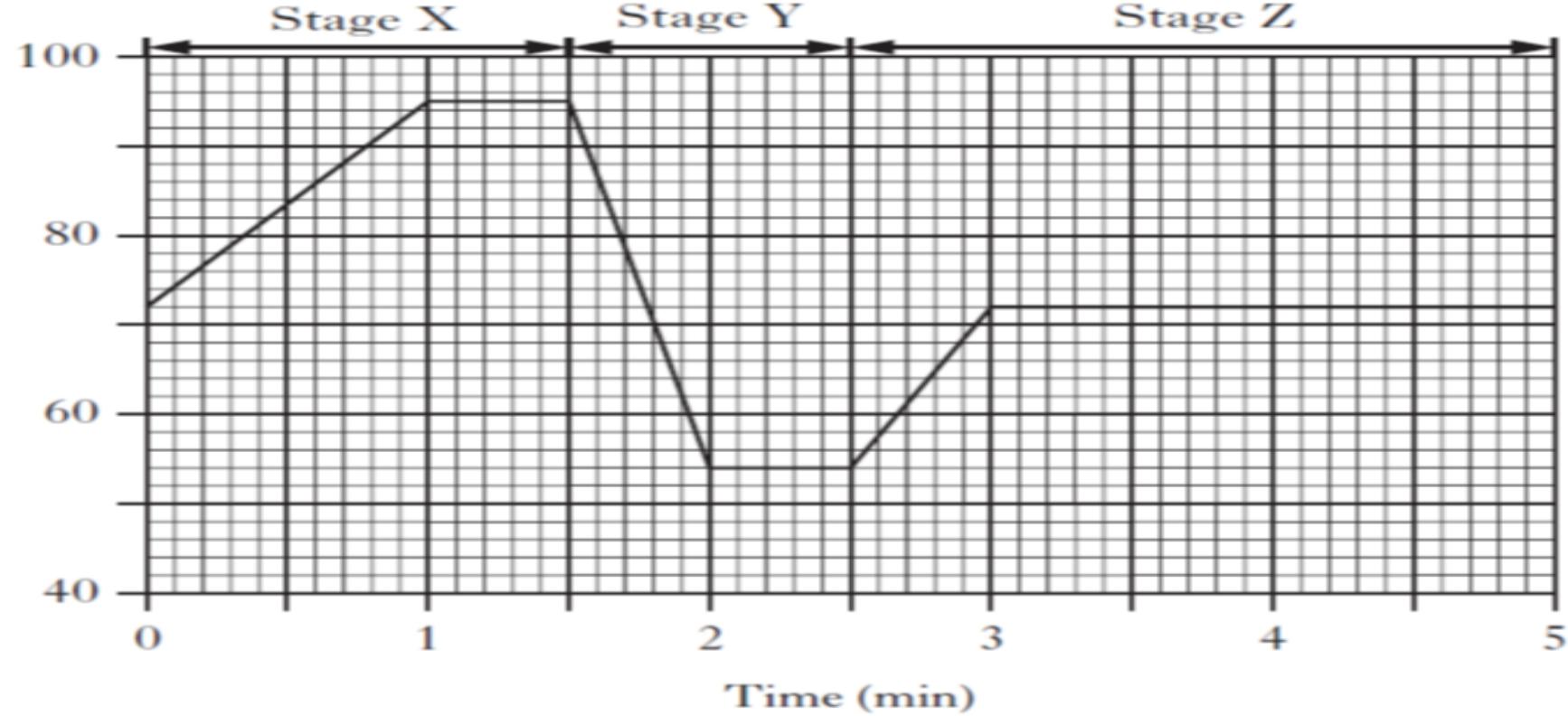




PCR enables exponential amplification of DNA from very small samples

Thought Questions

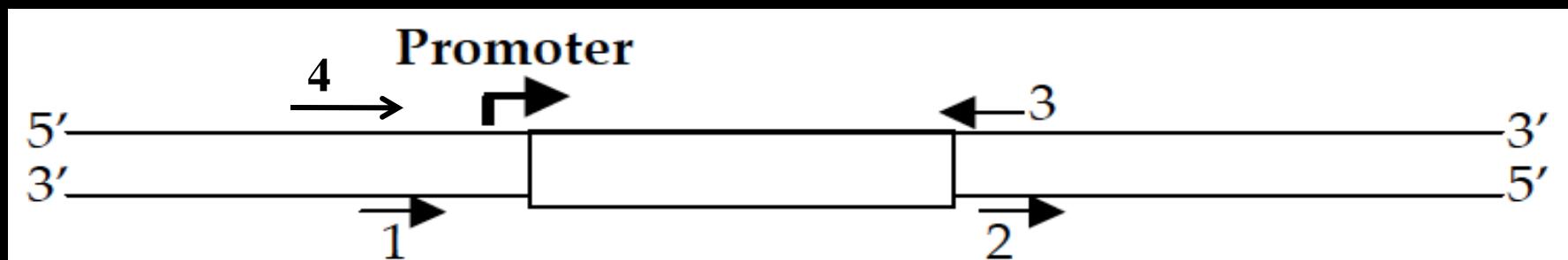
1. What reagents are needed for a successful PCR?
2. Give reasons for three temperature shifts during PCR
3. What would happen if you fail to denature the DNA?
4. What would happen if instead of using Taq you added a polymerase that works at temperature of 86C?
5. What would happen if you only add forward primer?
6. What would happen if you don't add dNTPs?



- a) Describe what happens to the DNA during stage X.
- b) State what happens to the primers during Stage Y.
- c) Suggest why the temperature is increased during Stage Z.
- f) A forensic scientist discovered a tiny spot of blood at a crime scene.
A sample taken from this spot contained 100 molecule of DNA.
The sample underwent PCR cycles for 40 minutes. Use the graph to calculate how many molecules of DNA would be present after this time.
- (ii) What process would then allow an individual to be identified from the DNA?

One way to make transgenic sheep is to have a gene of interest inserted into the sheep genome. You introduce a viral gene into the embryonic sheep cells in culture. To make sure, that the sheep born from the embryonic cells is a transgenic sheep with the viral gene, you decide to check the presence of the gene in cells isolated from the sheep. You have to PCR amplify your gene. You have four primers as diagrammed below, with your gene of interest shown in box.

- Which primer/s (from 1, 2, 3, 4 below) would you use for the PCR?
- Which of the following reagents would you add to your PCR mixture (which already contains the following- DNA polymerase, enzyme buffer and DNA primers). a. Genomic DNA from the transgenic sheep cell; b. Reverse transcriptase; c. dNTPs; d. DNA ligase.

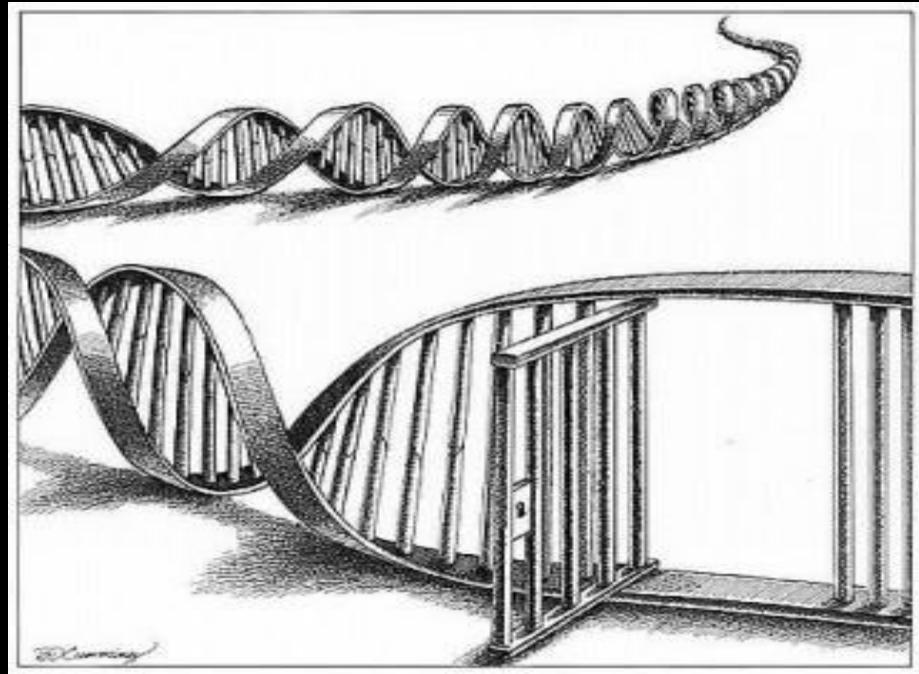


A scientist was trying to design an investigation using the polymerase chain reaction. The sequence he wished to amplify is part of a gene encoding a protein and is given below. The scientist decided he could only afford to use primers of 6 bases in length.

- a) Write down the sequence of the two primers he should order to be made.
- b) The scientist obtained two bands of amplified DNA. Explain under what circumstances two bands of amplified DNA would be obtained in the above experiment.



DNA Fingerprinting



The term DNA fingerprinting - or genetic fingerprinting - is applied to the scientific process whereby samples of DNA are collected, processed and used to match with other samples of DNA.

John Cole
©The Times-Picayune
SAVANTONIA
CagleCartoons.com





Beaten & Raped While In Prison

Contracts HIV while In Prison

Spends 22 Years In Prison

**IS THIS WORTH
9MILLION DOLLARS?**

TNN RAW & UNCUT NEWS

**Man Wrongly Convicted Spends 22 Years In
Jail, Raped Over 12 Times & Contracted HIV**

**Kirk Odom's family abandoned him for unsaid reasons;
he was robbed in his life**

For paternity and maternity tests



The chance of another person having
the same DNA fingerprint is one in
9,390,000,000

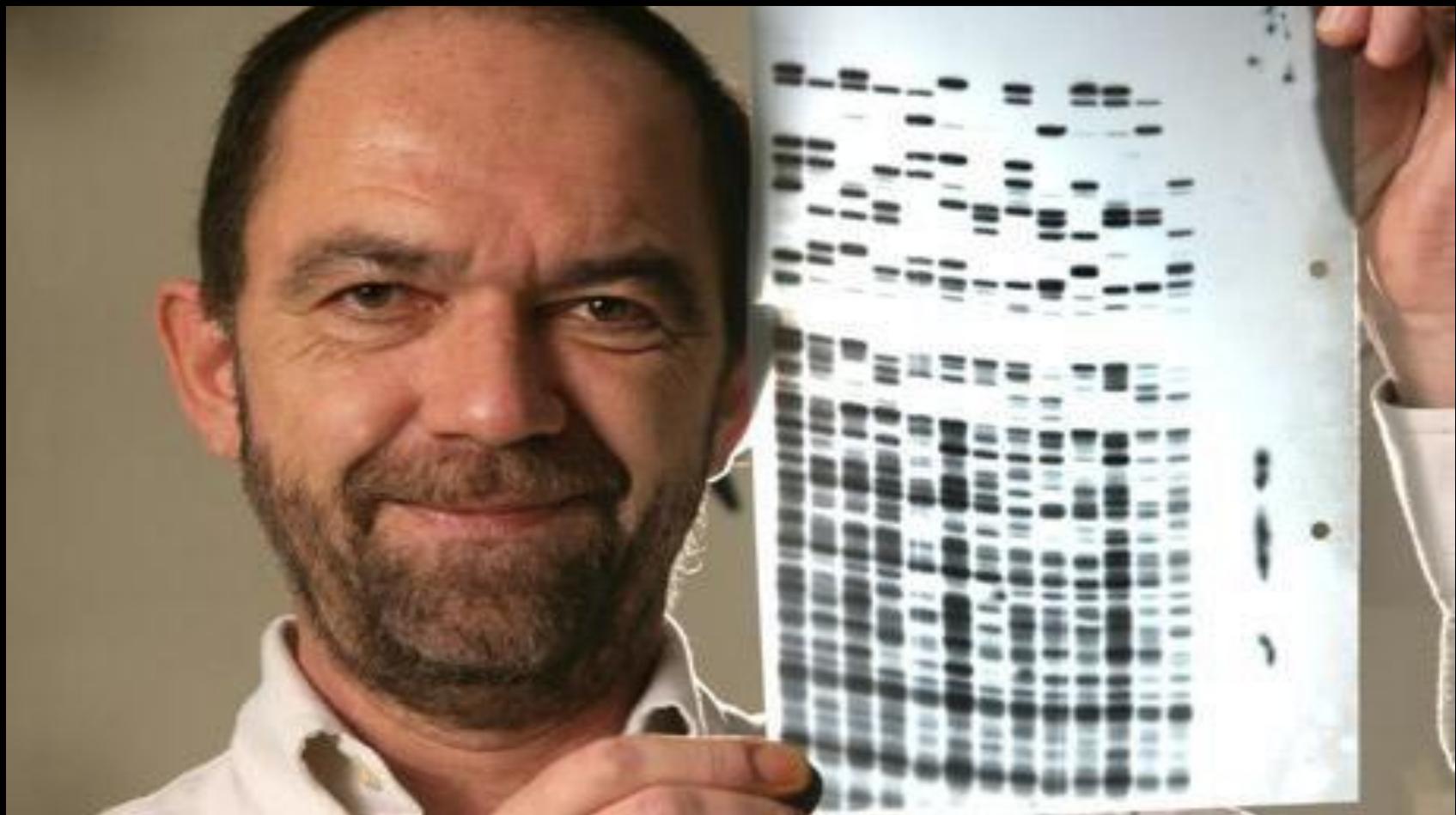
Our genetic differences are at the heart of one of the most fascinating paradoxes of the human condition: that

“we are all different, yet we are all the same”

---*Geneticist Mary-Claire King, 1993*

So where are we **Different**

Sir Alec Jeffreys in 1984



1996 – *Albert Einstein World Award of
Science*

STRs

- Short Tandem Repeats (**STRs**).
- Repetitive sequences of DNA (**2-5 nucleotides**) that are repeated various times in the genome.

.... GTTCAACCATCCATCCATGGCCAT

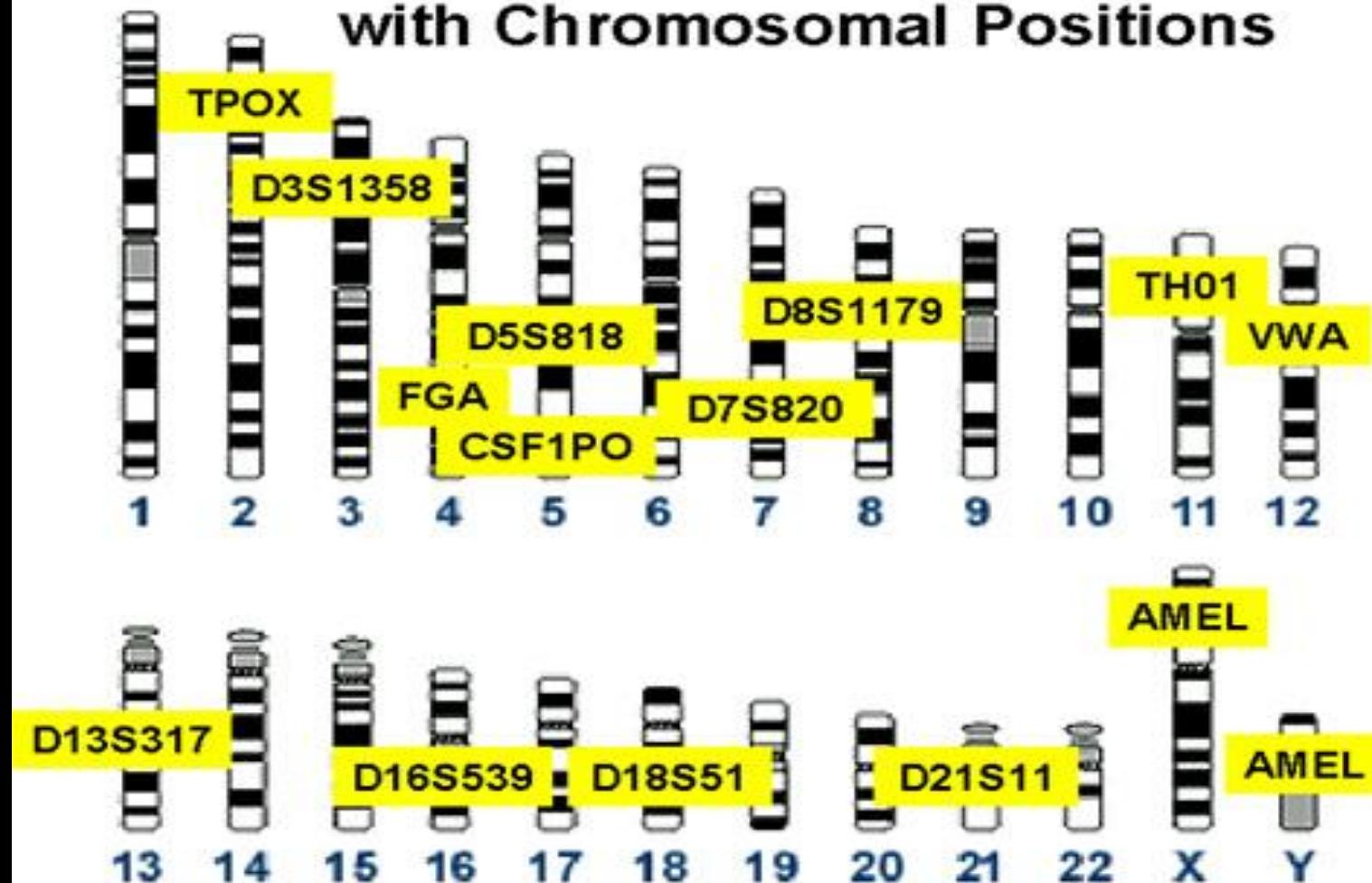
.... GTTCAACCATCCATCCATCCATGGCCAT

.... GTTCAACCATCCATCCATCCATCCATGGCCAT

.... GTTCAACCATCCATCCATCCATCCATCCATGGCCAT

Detecting a Crime Suspect

13 CODIS Core STR Loci with Chromosomal Positions

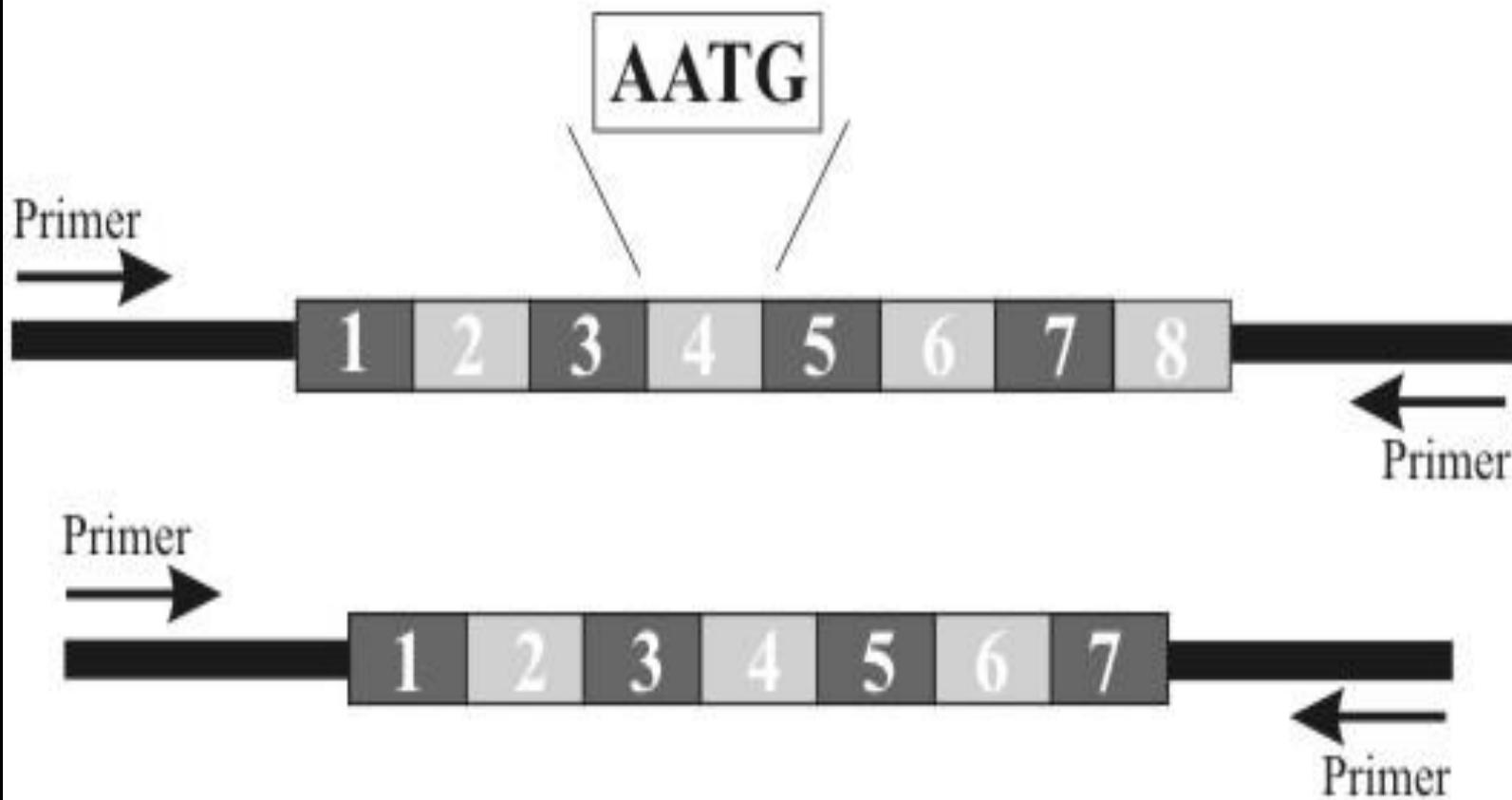




You are a private investigator and in a crime scene you find molecular evidences left behind by the criminal....how would you proceed with the case.....????

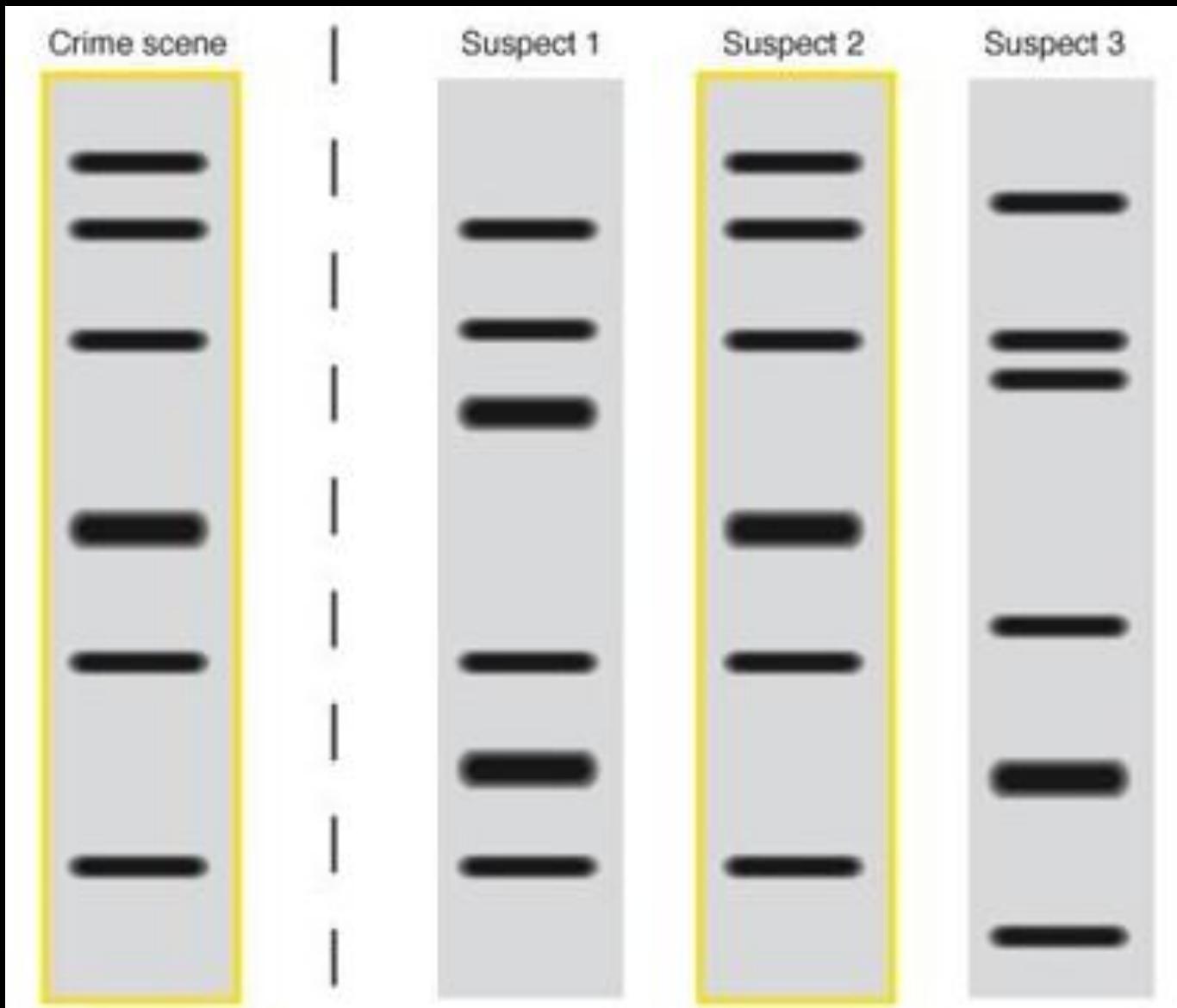
Amplification of Repeats

Short Tandem Repeats

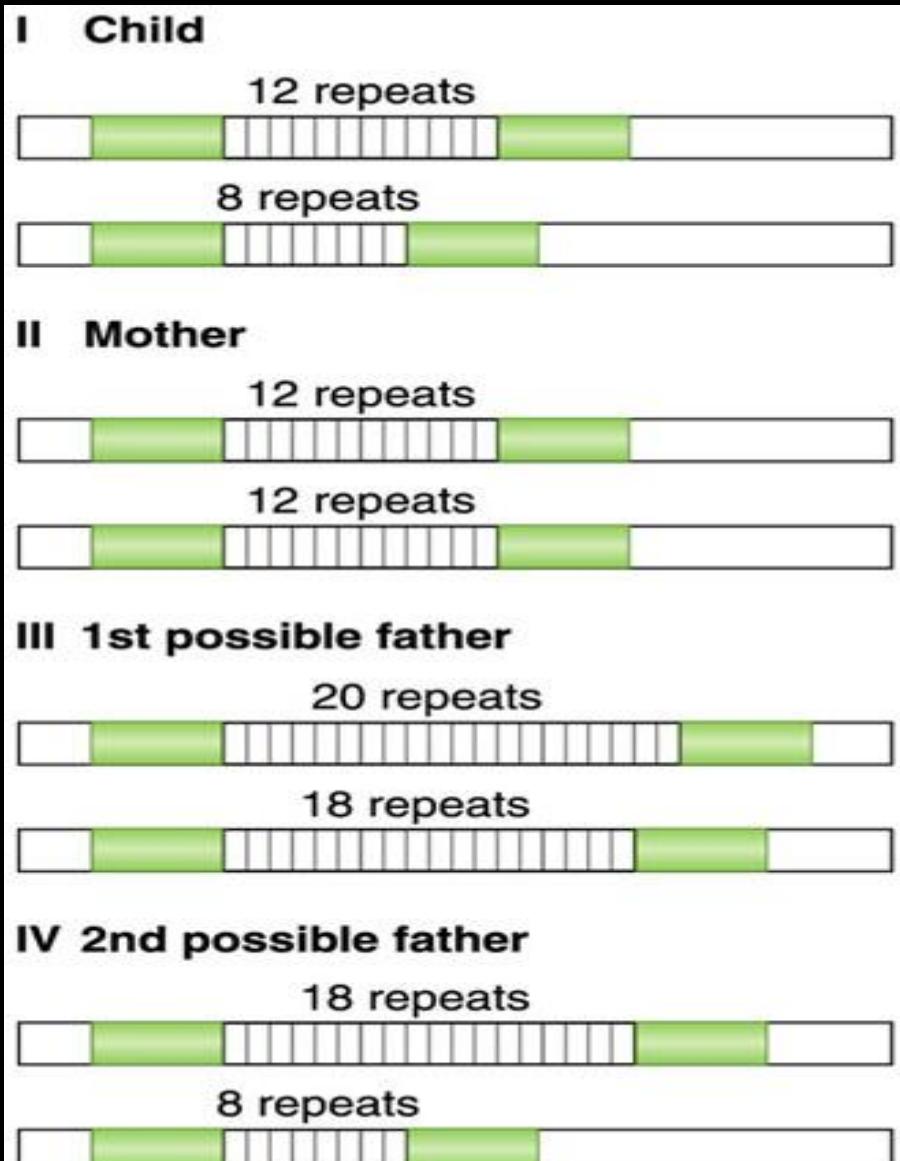


The flanking regions where PCR primers bind are constant

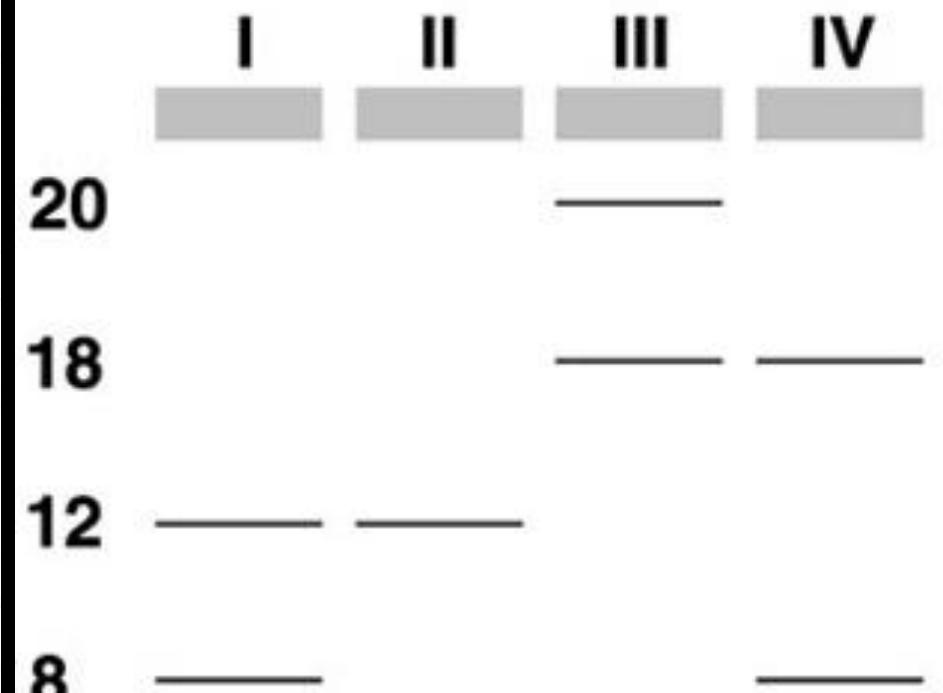
Gel Image of Amplified STRs



The mother died...and two persons claim the child..... identify the possible father



I - Child
II - Mother
III - 1st possible father
IV - 2nd possible father



Do Identical Twins Have Same/Different DNA?

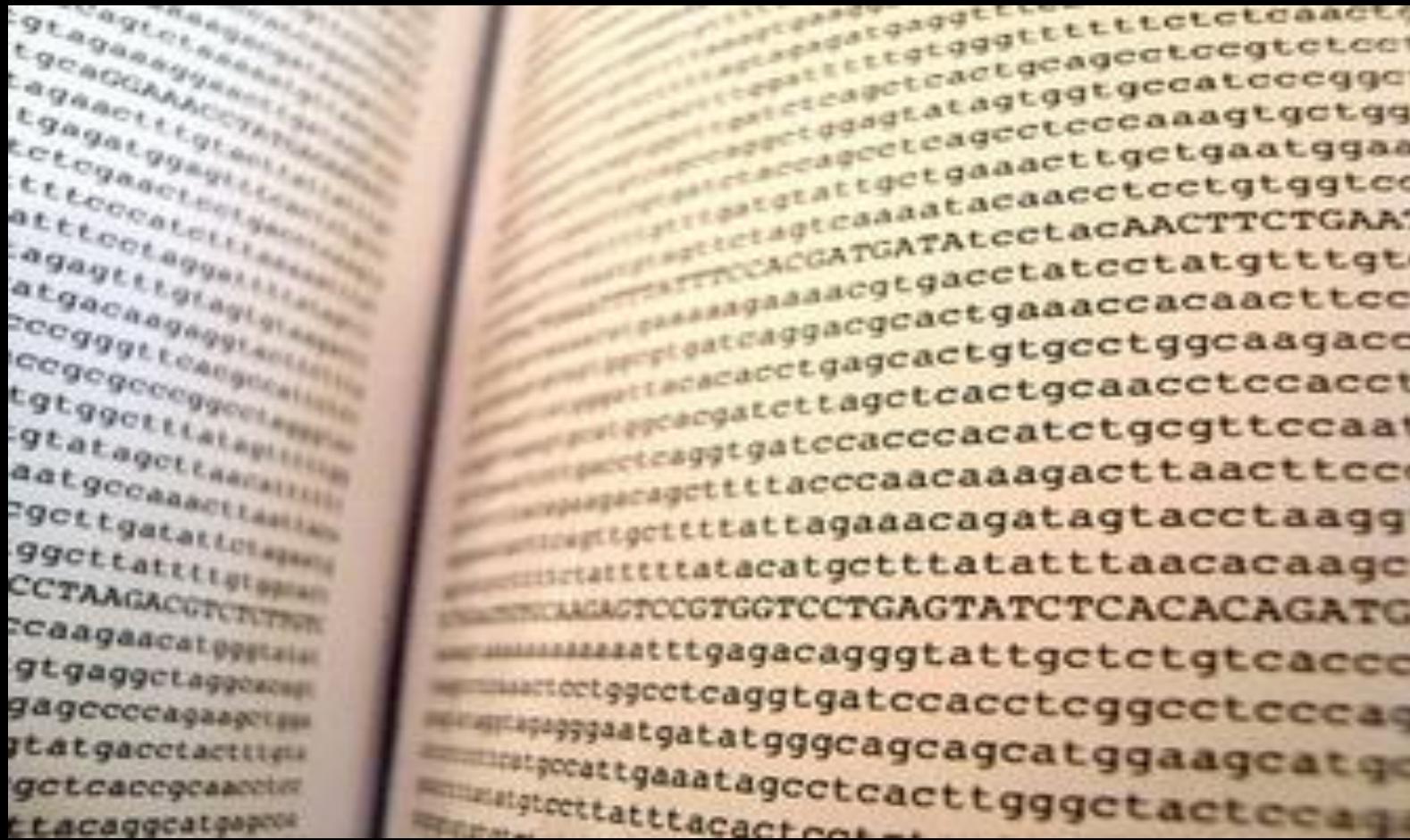


Some other useful applications of DNA profiling



The Age of Personalized Medicine is Around





The Human Genome Project

The team leader sums it up...

“When you have for the first time in front of you this 3.1 billion-letter instruction book that conveys all kinds of mystery about humankind, you can’t survey that going through page after page without a sense of awe.

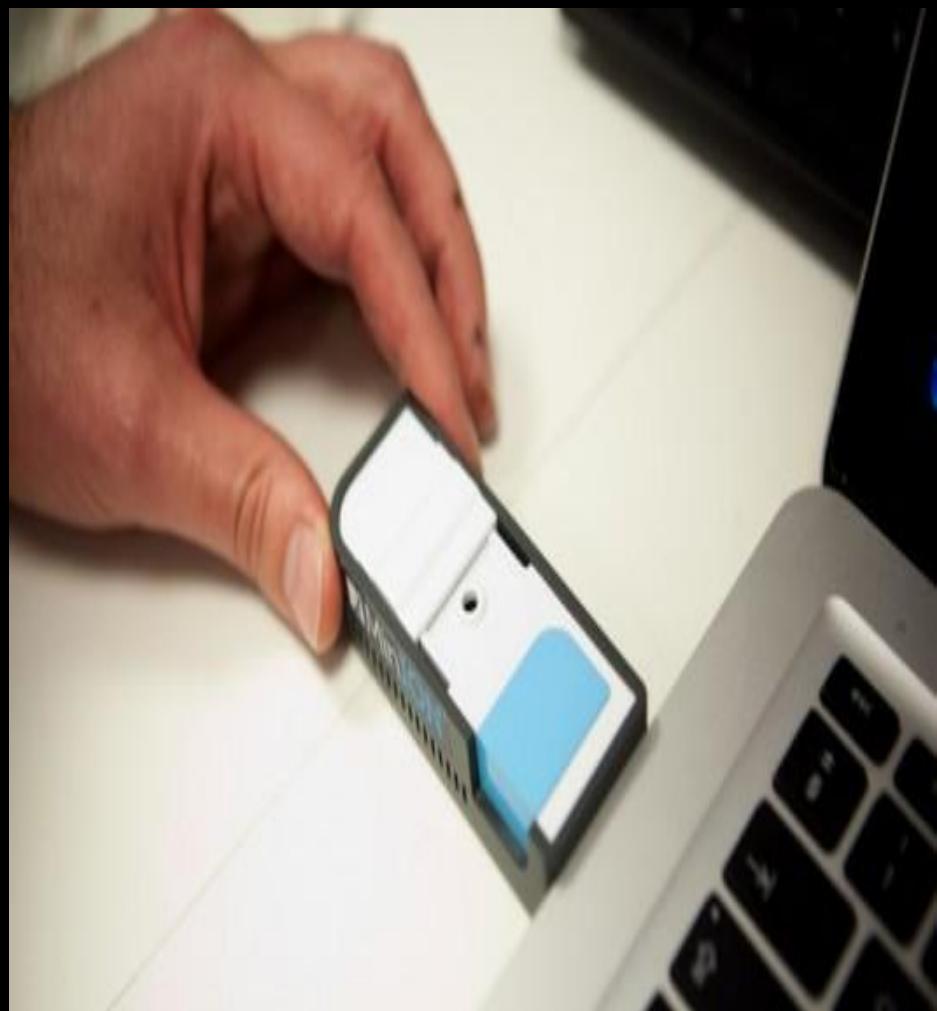
I can’t help but look at those pages and have a vague sense that this is giving me a glimpse of God’s mind.”

- Francis Collins
Director, HGP

**We are rapidly approaching the
day when an individual's
genome can be sequenced in a
matter of hours for less than
\$1,000**

Today and Tomorrow

Ion Proton DNA Sequencer



**What have been some of the
take home lessons from HGP?**

**1. The number of genes has
absolutely no relation to how
evolutionary advanced we
are...**



- The human genome project found that there are far fewer genes in the human genome than previously predicted.
- We have about the same number of genes as a **ROUND WORM** and only half as many as **RICE Plant**.

Table 12.1

Some Important Sequenced Genomes*

Organism	Year Completed	Size of Genome (in base pairs)	Approximate Number of Genes
<i>Haemophilus influenzae</i> (bacterium)	1995	1.8 million	1,700
<i>Saccharomyces cerevisiae</i> (yeast)	1996	12 million	6,300
<i>Escherichia coli</i> (bacterium)	1997	4.6 million	4,400
<i>Caenorhabditis elegans</i> (roundworm)	1998	100 million	20,100
<i>Drosophila melanogaster</i> (fruit fly)	2000	165 million	14,000
<i>Arabidopsis thaliana</i> (mustard plant)	2000	120 million	25,500
<i>Oryza sativa</i> (rice)	2002	430 million	42,000
<i>Homo sapiens</i> (human)	2003	3.0 billion	21,000
<i>Rattus norvegicus</i> (lab rat)	2004	2.8 billion	20,000
<i>Pan troglodytes</i> (chimpanzee)	2005	3.1 billion	20,000
<i>Macaca mulatta</i> (macaque)	2007	2.9 billion	22,000
<i>Ornithorhynchus anatinus</i> (duck-billed platypus)	2008	1.8 billion	18,500
<i>Prunus persica</i> (peach)	2013	227 million	27,900

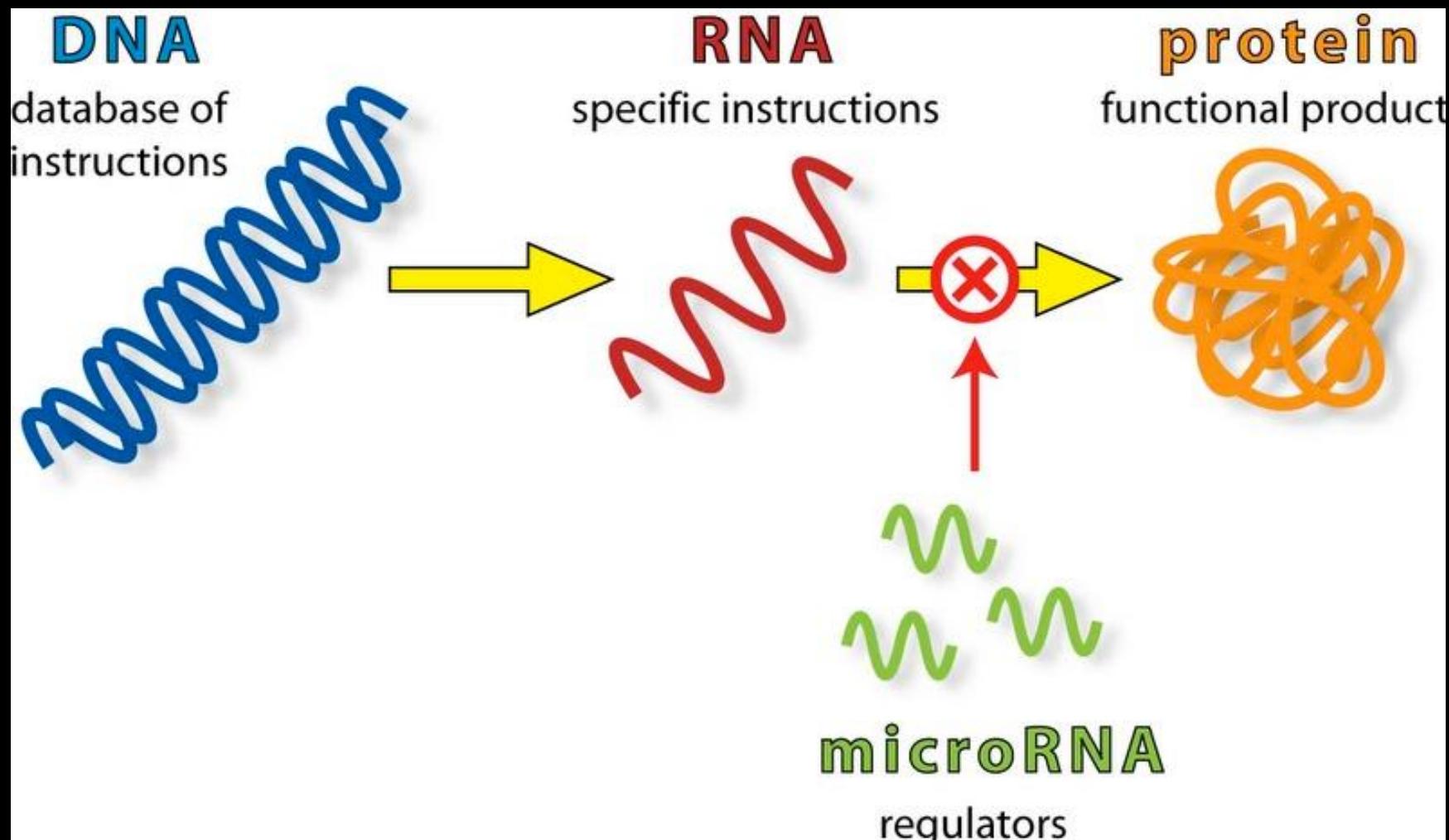
*Some of the values listed are likely to be revised as genome analysis continues.

2. There are about 20-25K genes and of these ~60% are alternatively spliced.

3. The coding sequences only make about 2% of our genome; 98% is non-coding.....

4. Lot of our DNA came from viruses that infected us thousands of years ago.

5. Human Genome can code for Non-coding RNAs-- miRNAs





NCBI

GenBank Overview

PubMed

Entrez

BLAST

OMIM

Books

Taxonomy

Structure

Search

Entrez

for

eat-4 elegans

Go

NCBI

SITE MAP

Submit to
GenBank

BankIt

Sequin

► What is GenBank?

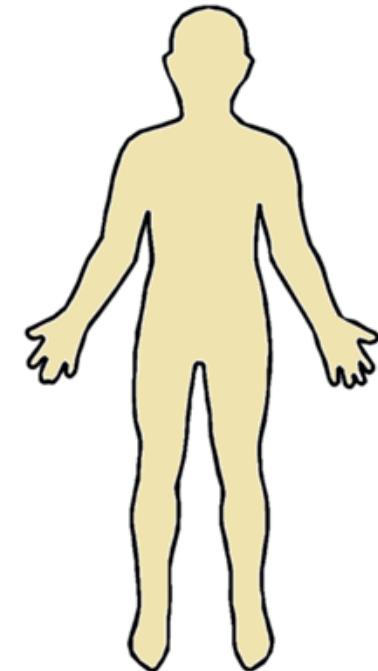
GenBank® is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences ([Nucleic Acids Research 2004 Jan 1;32\(1\):23-6](#)). There are approximately 37,893,844,733 bases in 32,549,400 sequence records as of February 2004 (see [GenBank growth statistics](#)). As an example, you may view the [record](#) for a *Saccharomyces cerevisiae* gene. The complete [release notes](#) for the current version of GenBank are available. A new release is made every

- The DNA sequences are deposited in GenBank, a database that is available to anyone via the Internet.
- You can browse it yourself at the website for the National Center for Biotechnology Information (ncbi.nih.gov).

**But still today we are nowhere
into understanding our
genome.....**



?????



**Can we read the genome and
build or assemble a human
being???**

**Are Biotechnologists playing
“God”?**

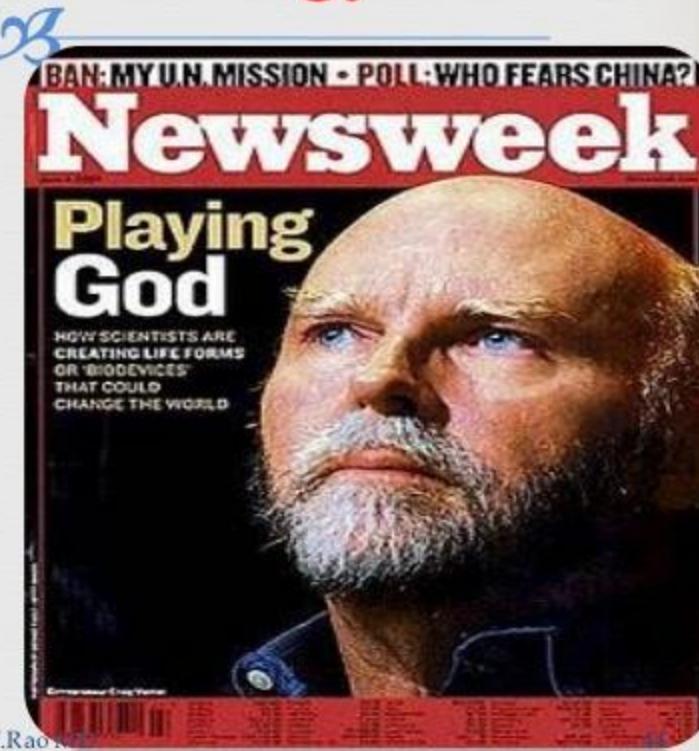
World's First Synthetic Life:

Wh

em,"

Craig venter creates revolution in Synthetic Biology

Craig Venter's team (and the associated [paper](#) in Science) that they have successfully synthesized the complete genome of the bacterium *Mycoplasma genitalium* is an important step towards achieving what is becoming known as "synthetic biology". By constructing complete DNA sequences from scratch, the door is being opened to transforming common laboratory chemicals into new living organisms; that are engineered with specific purposes in mind. And perhaps not surprisingly, this manipulation of DNA at the nan scale is increasingly being seen as part of the "nanotechnology revolution".



Research
constructed
cell. The team synthesized 1.08 million base pair (2010).
He could boot up the system.

Are we at an age where we can resurrect Dinosaurs



JANUARY 14, 2013

Joe Klein:
The CIA's
Afghan Disaster

Yemen: The
New Center
Of Terror

Why the Recession
Hasn't Been Cool
To Teens

TIME

WHY YOUR DNA ISN'T YOUR DESTINY

The new science of epigenetics reveals how the choices you make can change your genes—and those of your kids

BY JOHN CLOUD

\$4.99 US \$5.99 CAN



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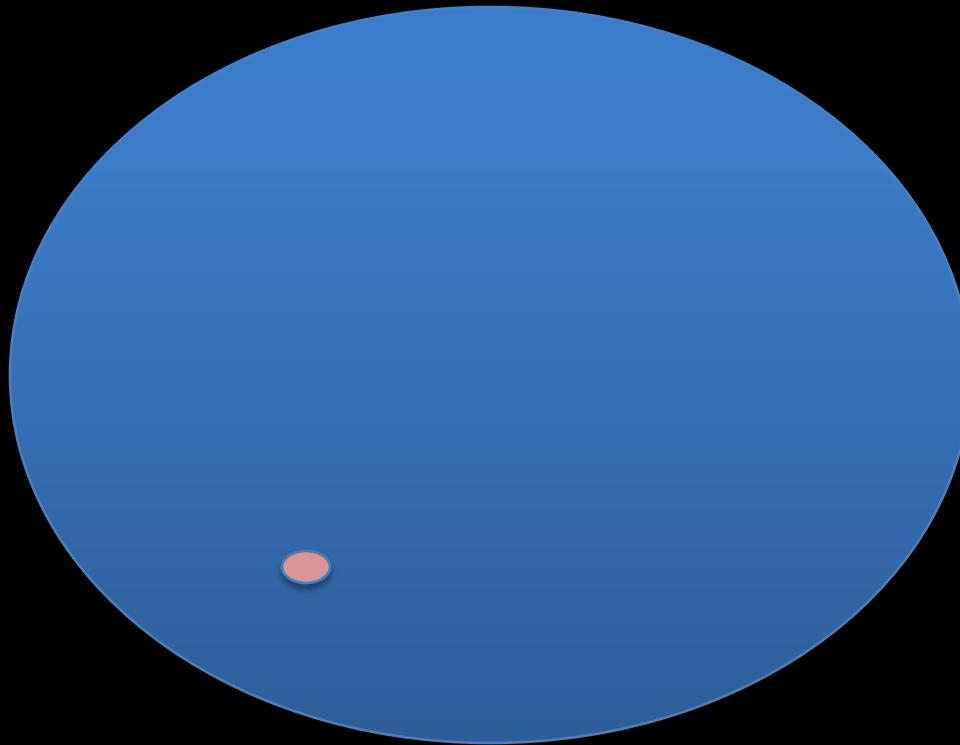
www.time.com

Have disease

Take pill

Kill something

Number of human physiological reactions-----probably....a million

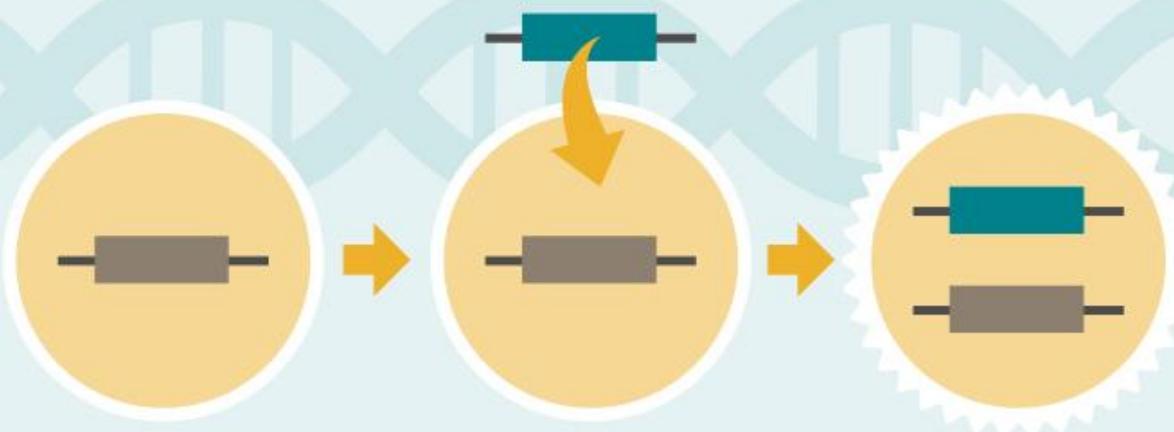


Currently targeted by medicine---
250

**Could your medicine be a cell or a
gene, and not a pill????**

What is Gene Therapy?

Gene therapy gives patients a healthy version of a defective gene



cell has
defective gene

healthy gene
is introduced

cell function
is restored

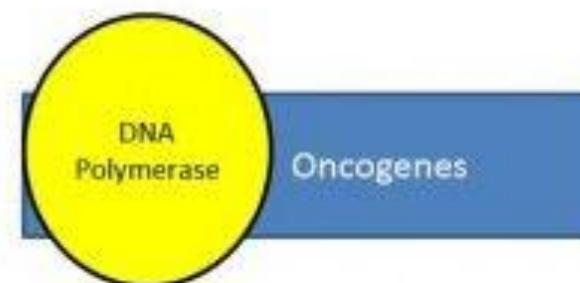


In order for cancer to occur

Tumor Suppressor Genes (TSGs)
are turned 'OFF'



Oncogenes are turned 'ON'





Normal genes
(regulate cell growth)

Tumor suppressor genes



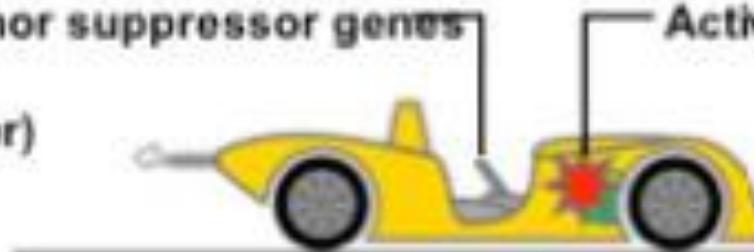
CANCER



1st mutation
(susceptible carrier)

Tumor suppressor genes

Active oncogene

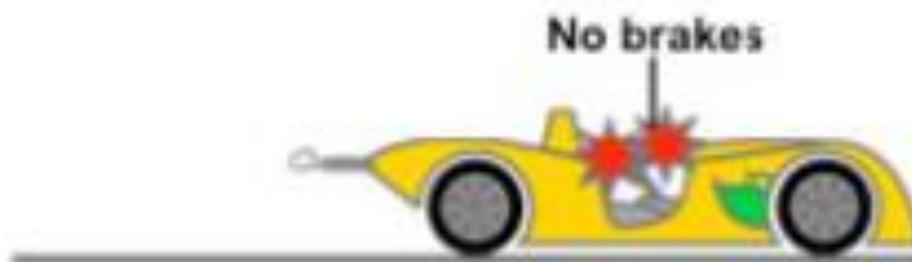


CANCER



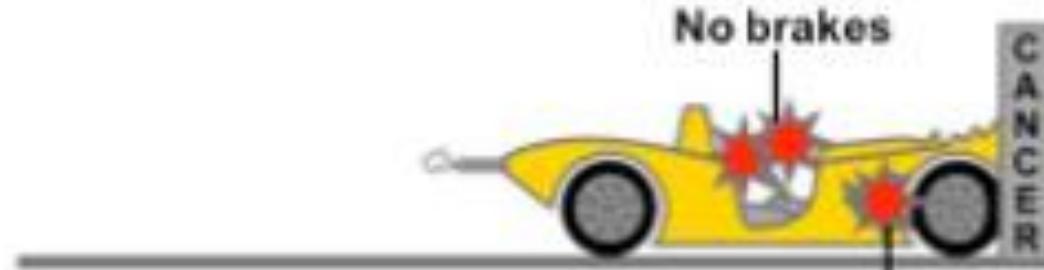
2nd mutation
or loss (leads
to cancer)

No brakes



CANCER

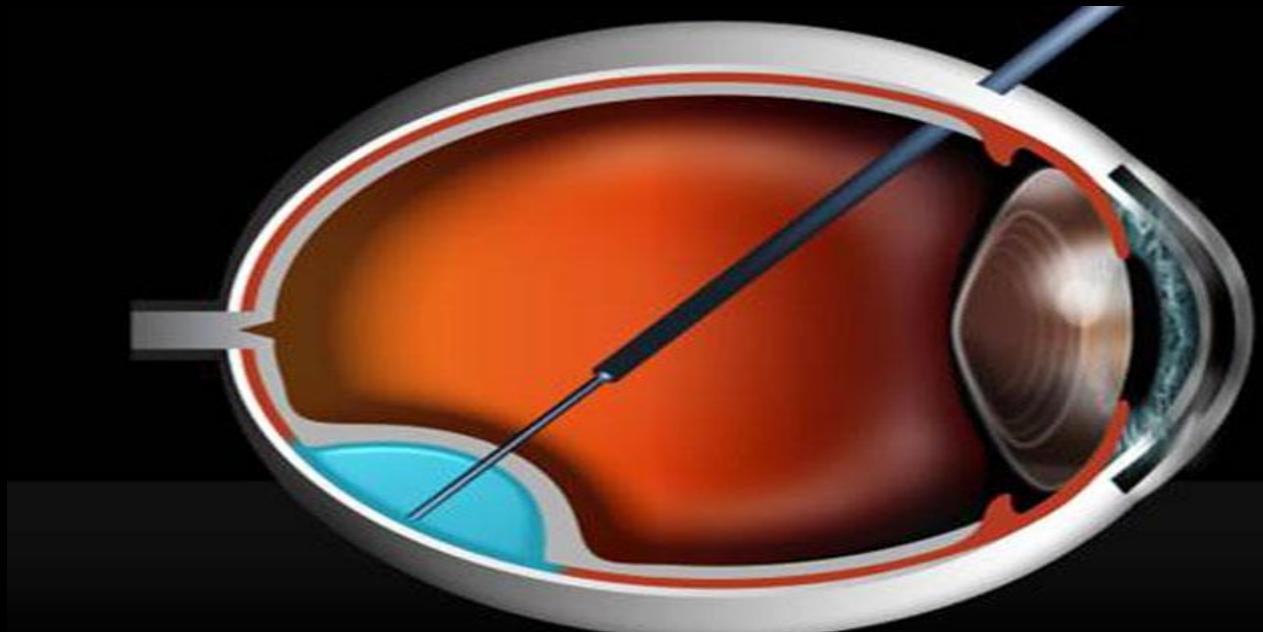
No brakes



Active oncogene

CANCER

Human Gene Therapy



- In 2009, a trial was conducted that focused on a form of blindness linked to a defect in a gene.
- The researchers found that a single injection of a virus carrying the normal gene into one eye of affected children improved vision in that eye, sometimes enough to allow normal functioning.