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http://www.icgeb.org

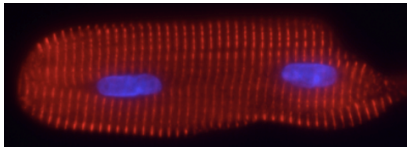
International Centre for Genetic Engineering and Biotechnology (ICGEB)

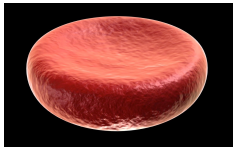
Trieste

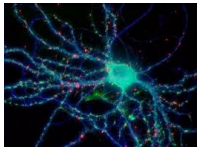
Genetic Engineering

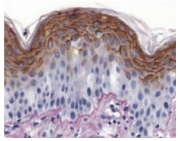
Adriatic sea

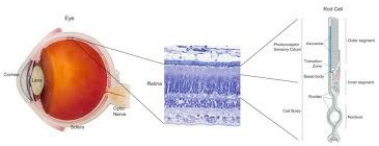
Our body is made up of cells


Cardiomyocyte

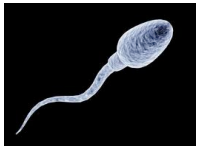

Red blood cell

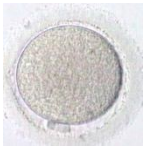

Neuron

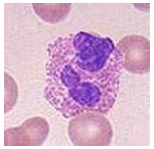

Keratinocytes

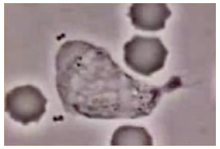

Eye photoreceptors

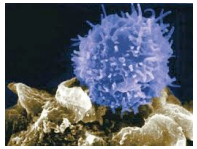
Gut epithelial cells


Spermatozoon

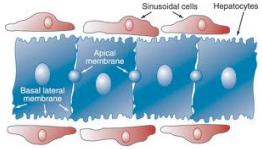

Oocyte


Granulocyte

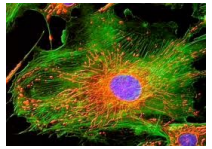

Macrophage


T-lymphocyte


Respiratory tract ciliated cells



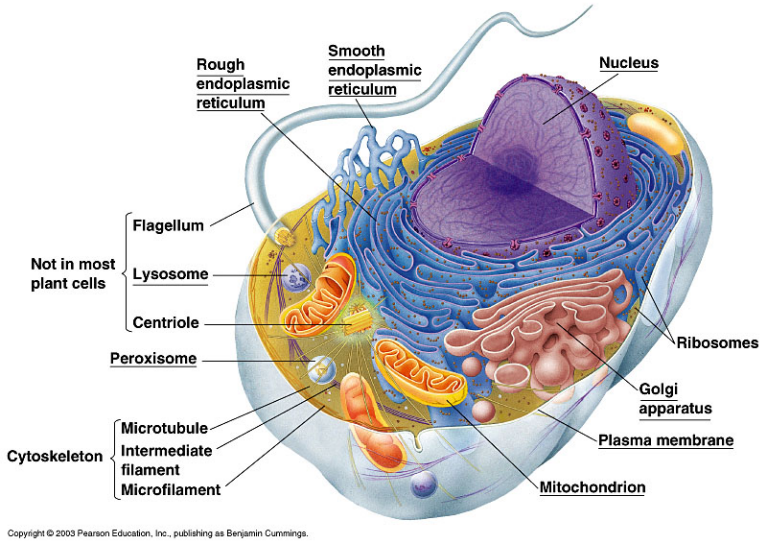
Hepatocytes


Endothelial cells

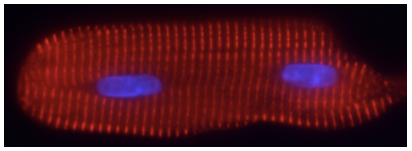
The human body:

~1x10^{14} (100 trillion) cells

200+ different cell types

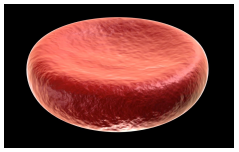


Cells' functions are exerted by proteins



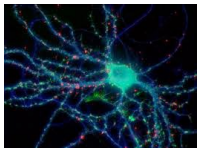
Cardiomyocyte

Actin and myosin,
contraction



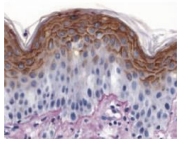
Red blood cell

Hemoglobin,
oxygen transport



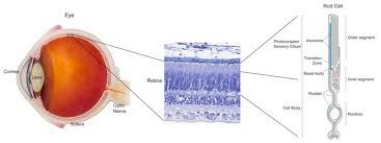
Neuron

Receptors for neurotransmitters,
movement and sensitivity



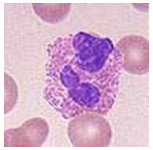
Keratinocytes

Keratin,
skin barrier



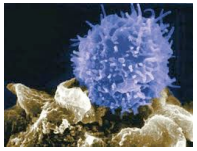
Eye photoreceptors

Opsin,
light sensor



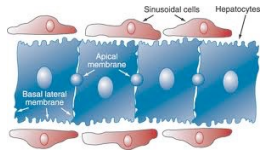
Granulocyte

Various enzymes,
destruction of microorganisms



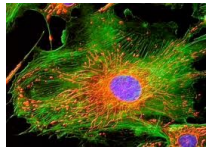
T-lymphocyte

Perforin,
killing of virus-infected cells



Hepatocytes

Various enzymes,
metabolism



Endothelial cells

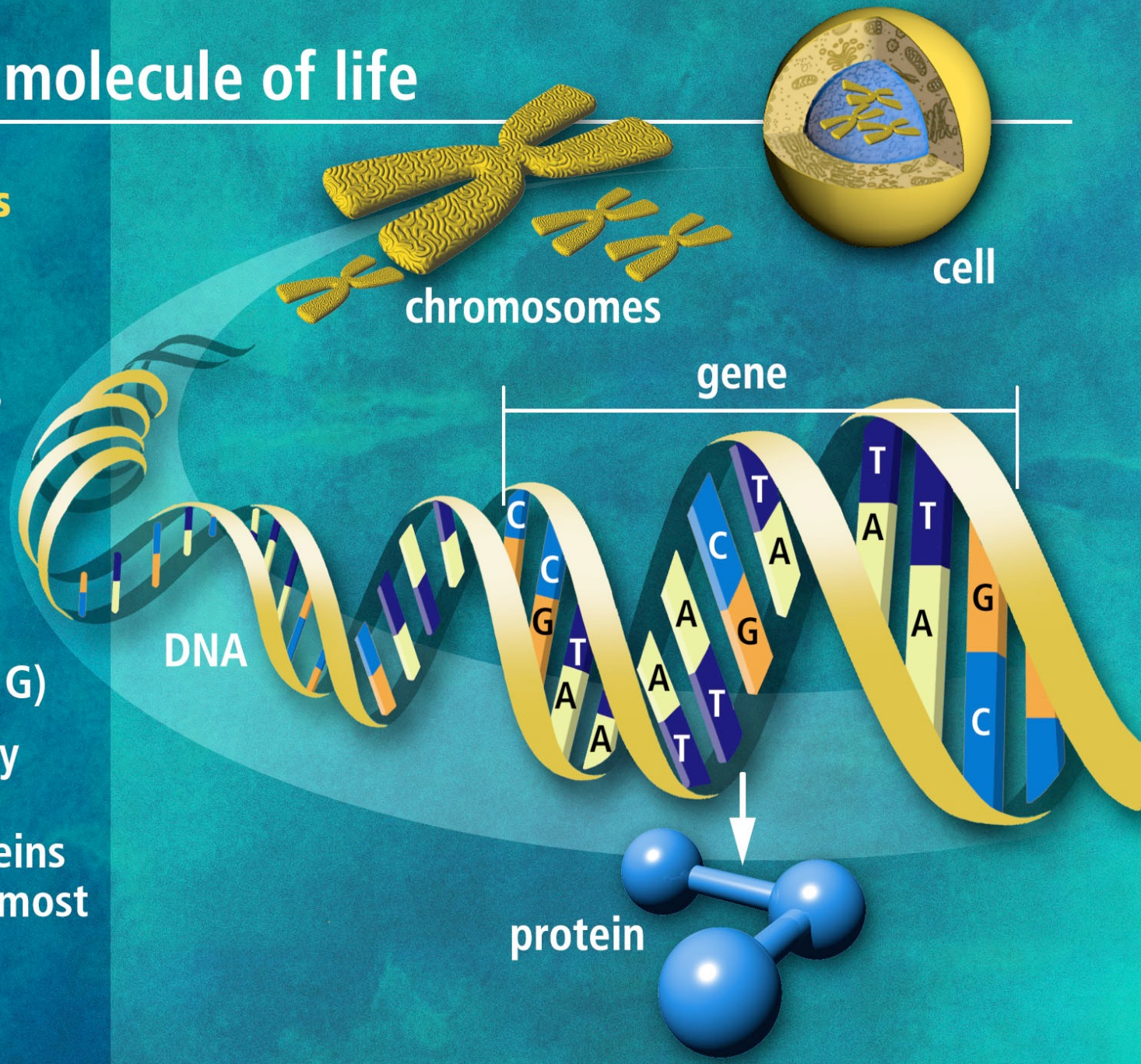
Junctional proteins,
vessel impermeability

DNA the molecule of life

Trillions of cells

Each cell:

- 46 human chromosomes
- 2 meters of DNA
- 3 billion DNA subunits (the bases: A, T, C, G)
- Approximately [redacted] genes code for proteins that perform most life functions



The human genome

Number of chromosomes:	46	
Size of the human genome:	~3x10^9 bp	
Number of genes:	~20.000	
Genes:	30%	
Extragenic DNA	70%	
Coding regions	<2%	(!)
Non coding regions	>98%	

The main genomes sequenced before the human genome

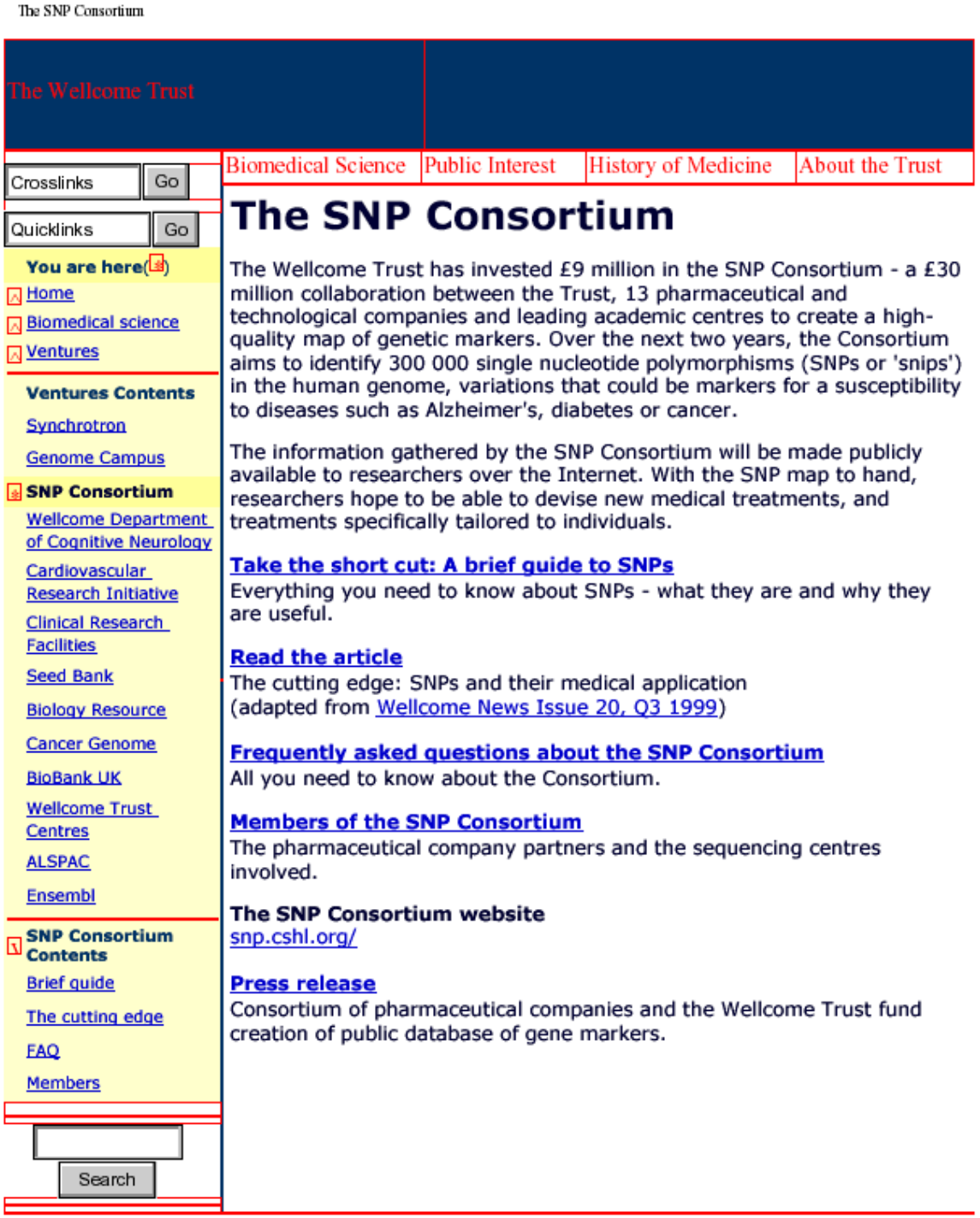
YEAR	ORGANISM	SIZE	NUMBER OF GENES
1984	Bacteriophage lambda	0.049	
1991	Smallpox virus	0.186	187
1995	Haemophilus infulenzae	1.8	1,740
1996	Saccharomyces cerevisiae	12.1	6,000
1997	Helicobacter pylori	1.67	1,590
1997	Escherichia coli	4.64	4,288
1998	Coenorabditis elegans	97	19,099
2000	Drosophyla melanogaster	180	13,061
2000	Pseudomonas aeruginosa	6.3	6,000 ?
2000	Arabidopsis thaliana	100	25,000
2001	Homo sapiens sapiens	2,910	26-38,000
	Mus musculum		
	Plasmodium falciparum (chr 2 and 3)		

The human genome

Number of chromosomes: 46
Size of the human genome: ~3x10^9 bp
Number of genes: ~20.000

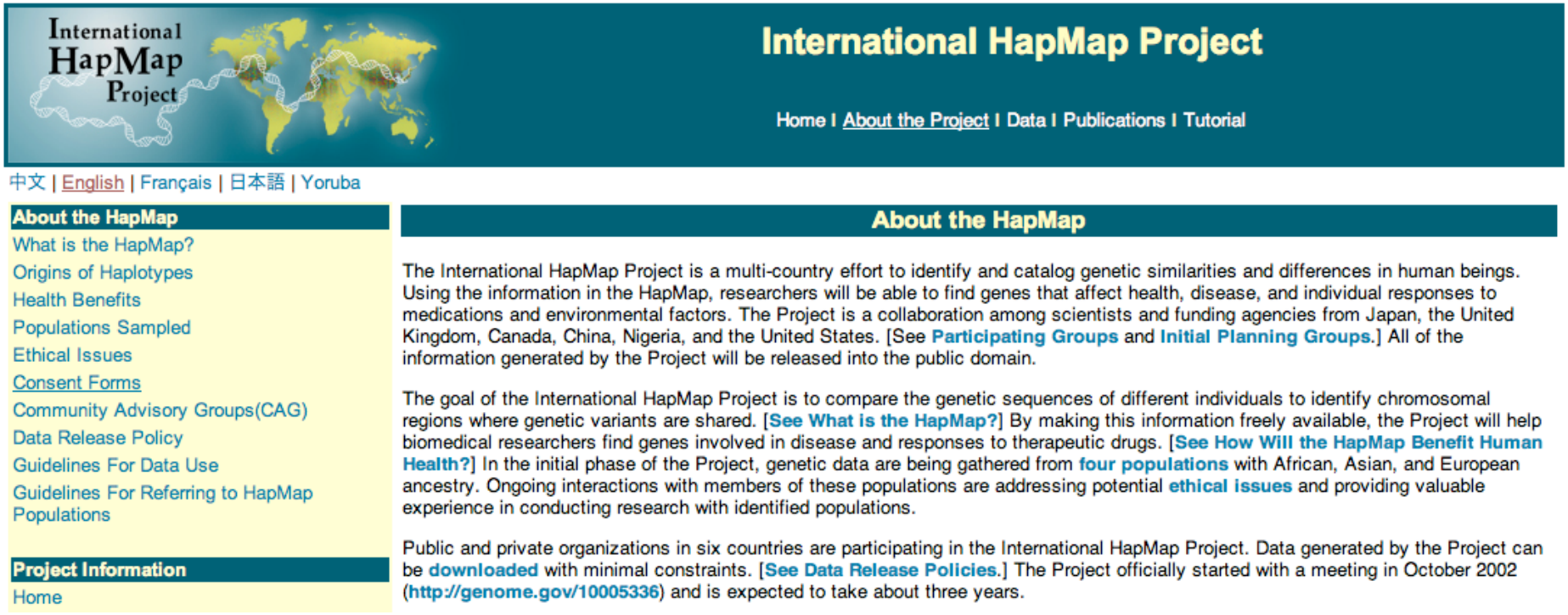
Genes: 30%
Extragenic DNA 70%
Coding regions
Non coding regions

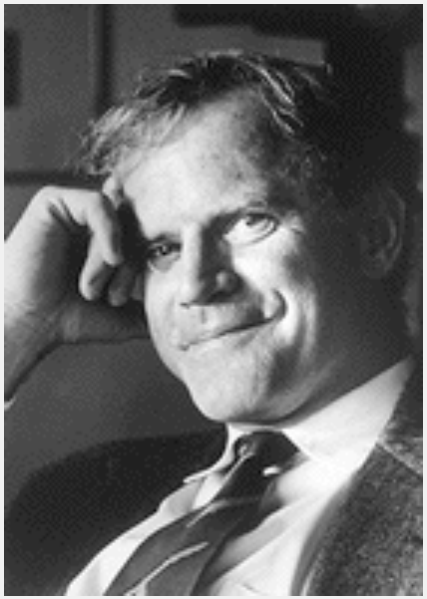
(!)



Confrontando il DNA di due individui sani si riconoscono circa 3 milioni di differenze (1 nucleotide ogni mille)

Approximately 3 x10^6 differences exist between the genomes of two healthy individuals (1 every 1000 nucleotides)

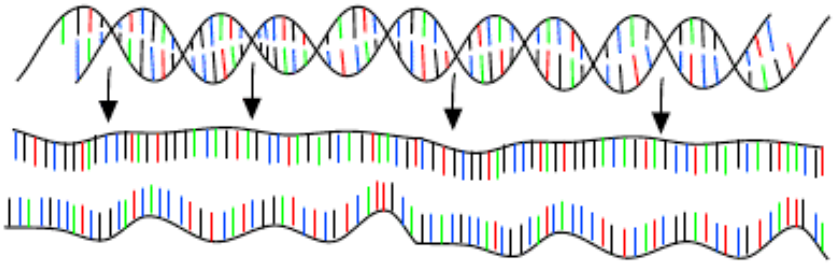




Kary B. Mullis

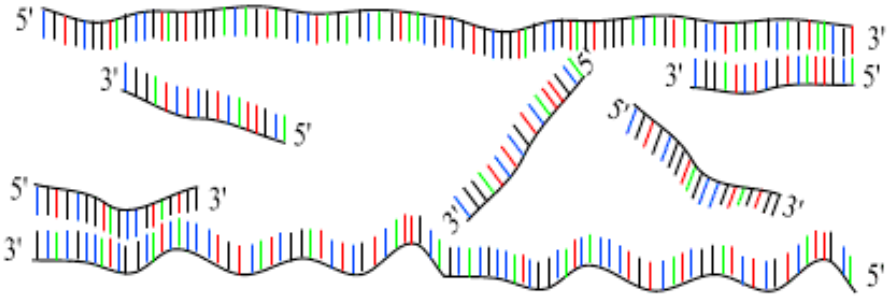
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

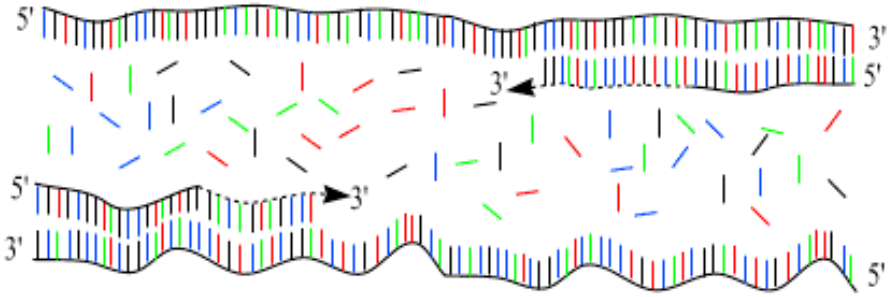
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

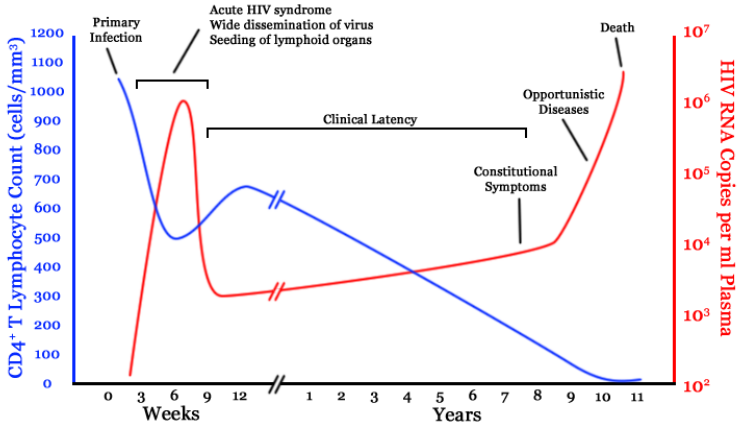
2 minutes 72 °C

only dNTP's

(Andy Vierstraete 1999)

Applications of PCR:

1. Detection of pathogens



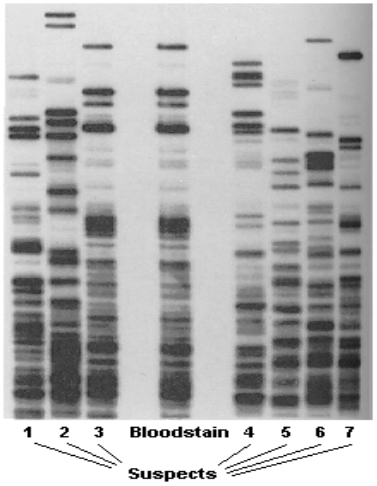
2. Diagnosis of genetic diseases

3. Identification of criminals, forensic medicine, paternity test

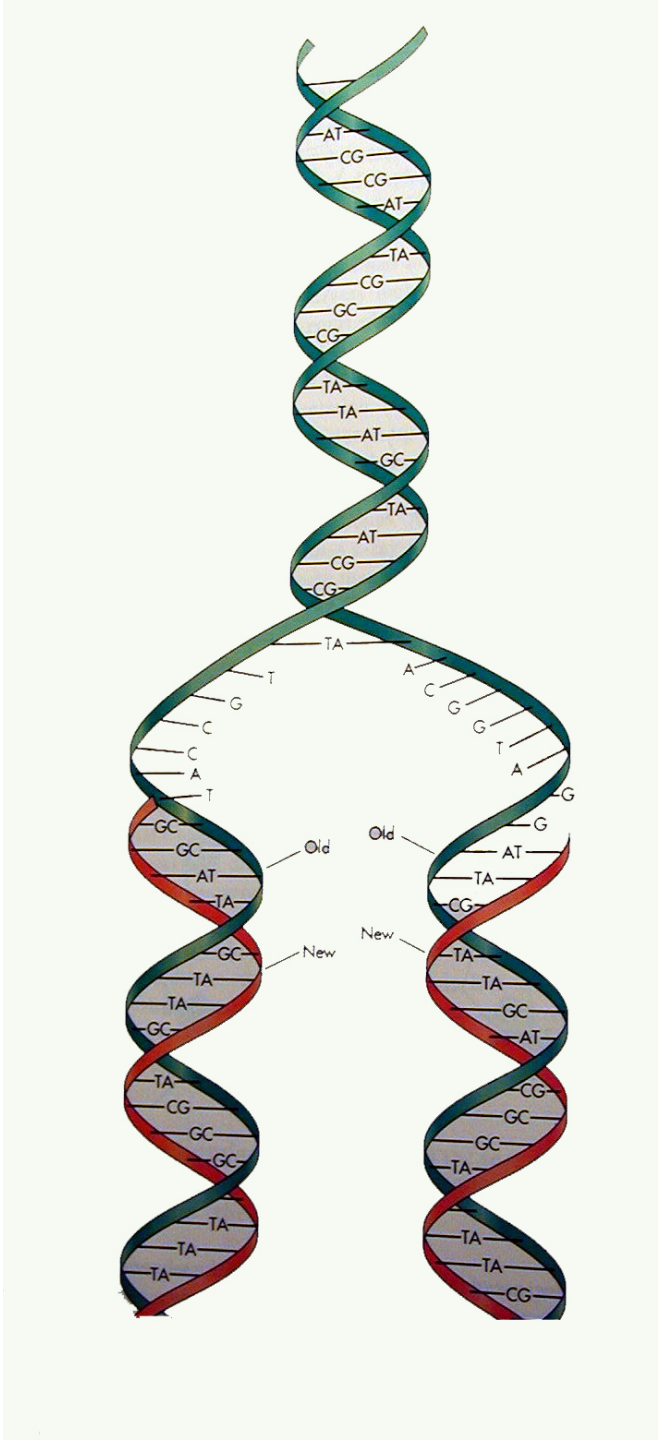
4. Monitoring gene expression

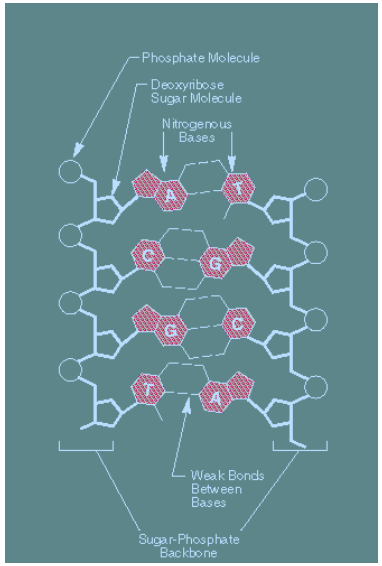
5. Evolutionary tracing

6. DNA cloning



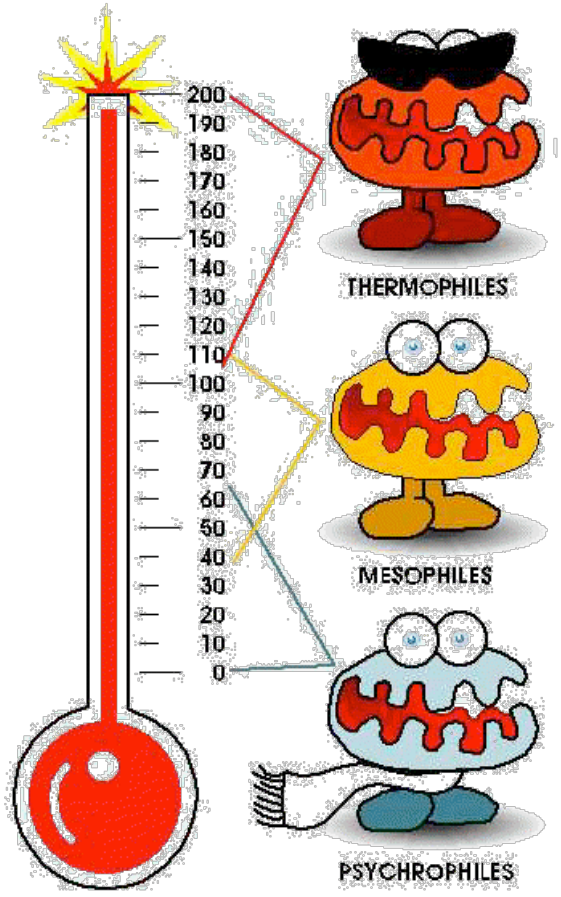
The molecular bases of DNA replication

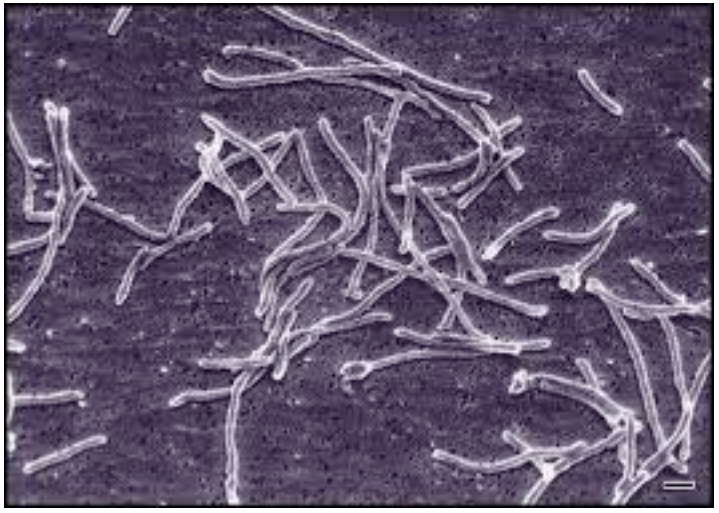




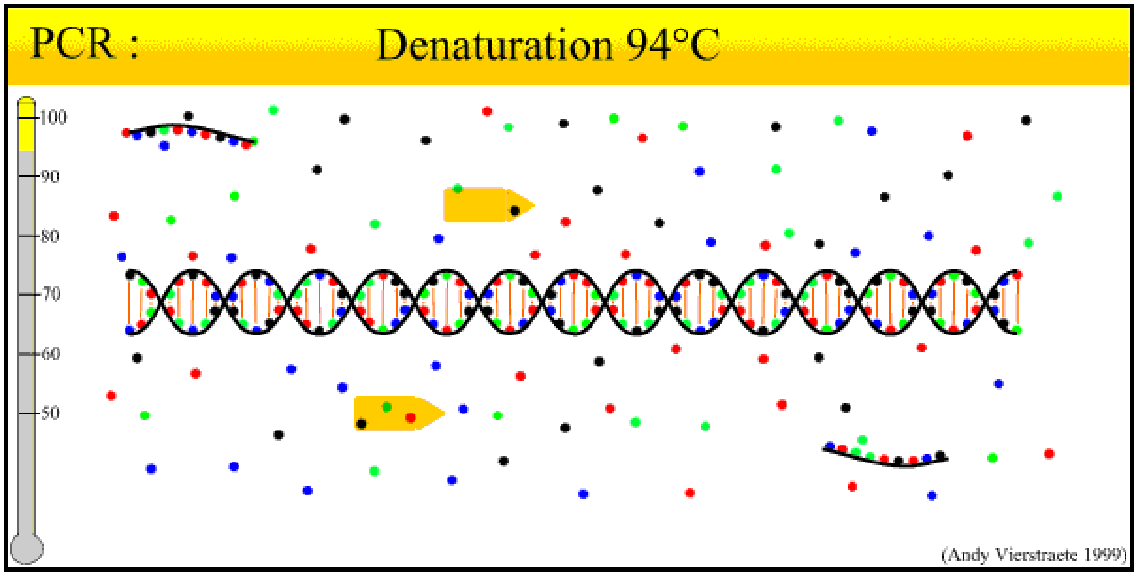
Taq polymerase is a DNA polymerase derived from Thermus Aquaticus

Thermus Aquaticus is a Gram Positive bacterium that is classified under a group called thermophiles. Thermophiles are defined as organisms that thrive and reproduce at temperatures that are above 45 Degrees Celsius. Specifically, Thermus Aquaticus optimally thrives and reproduces at 70 degrees celsius.



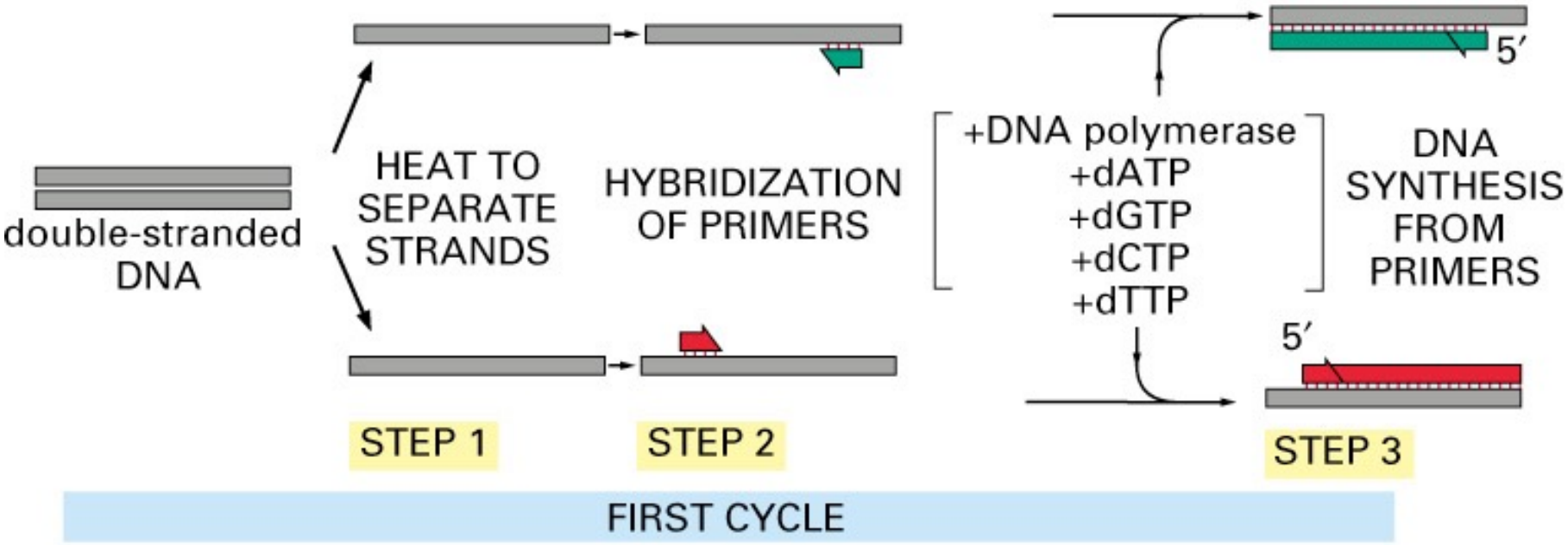


Polymerase Chain Reaction (PCR)

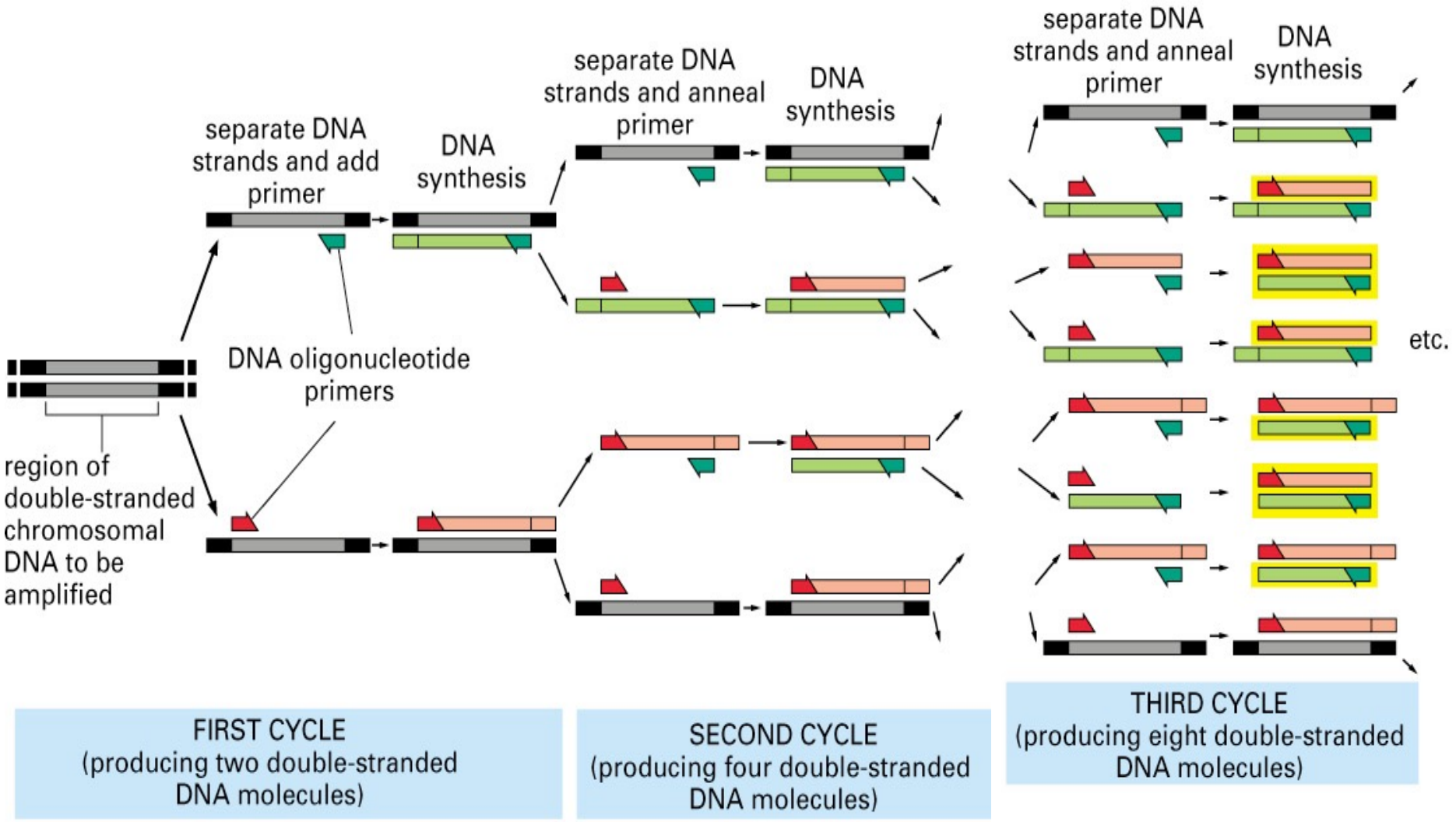


- DNA is denatured
- Primers attach to each strand
- A new DNA strand is synthesized behind primers on each template strand

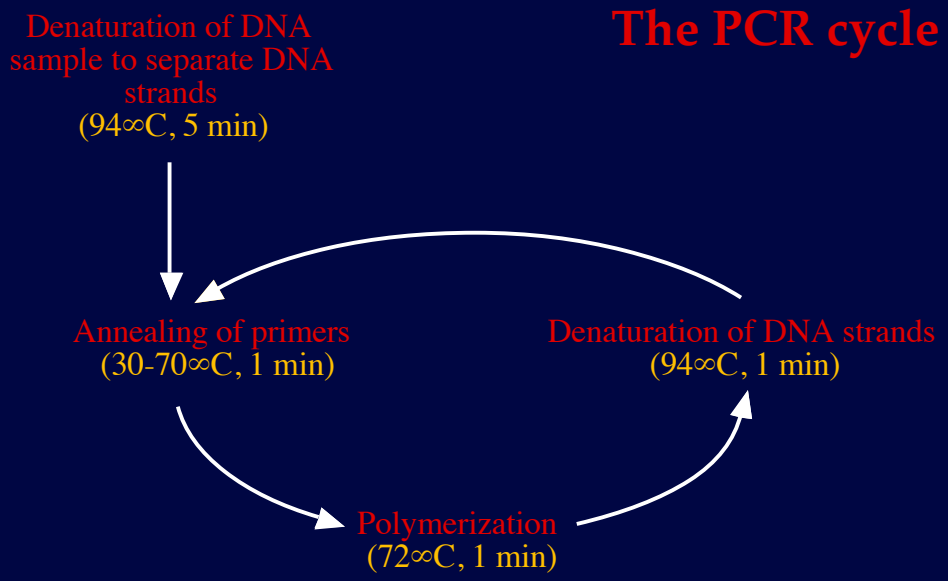
PCR amplification



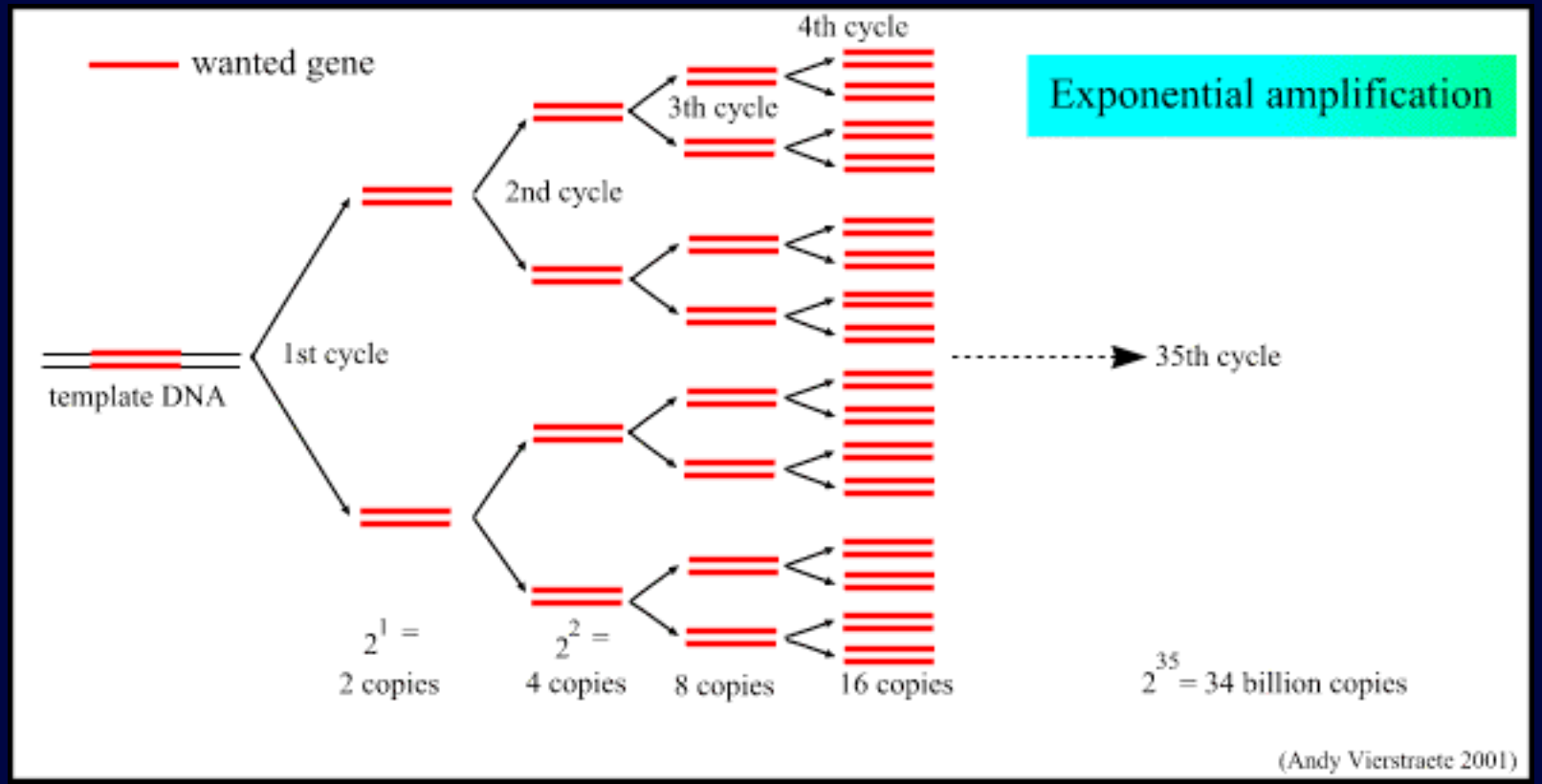
PCR cycles

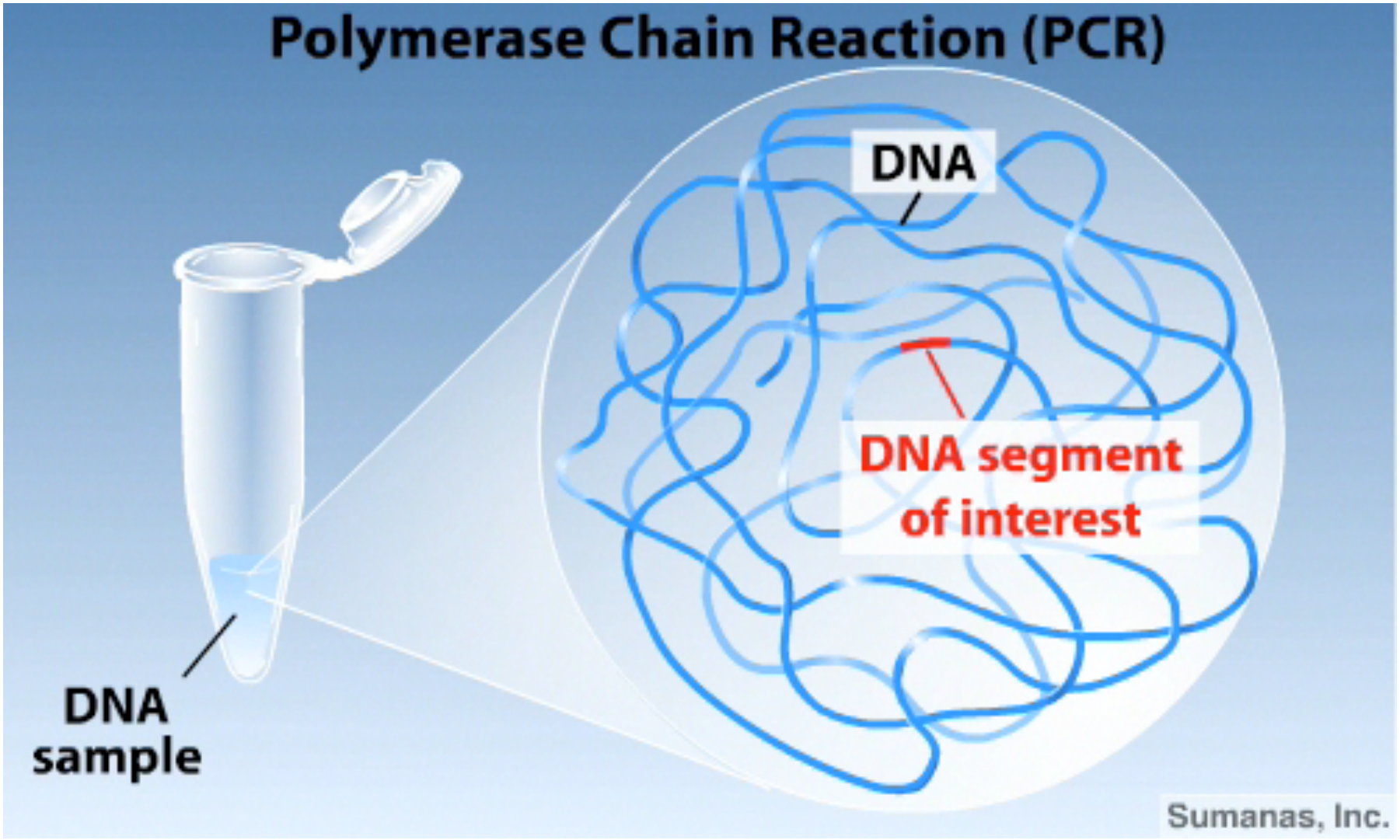

separate DNA strands and add primer
DNA synthesis
separate DNA strands and anneal primer
DNA synthesis
separate DNA strands and anneal primer
DNA synthesis
DNA oligonucleotide primers
region of double-stranded chromosomal DNA to be amplified
etc.
FIRST CYCLE
(producing two double-stranded DNA molecules)
SECOND CYCLE
(producing four double-stranded DNA molecules)
THIRD CYCLE
(producing eight double-stranded DNA molecules)

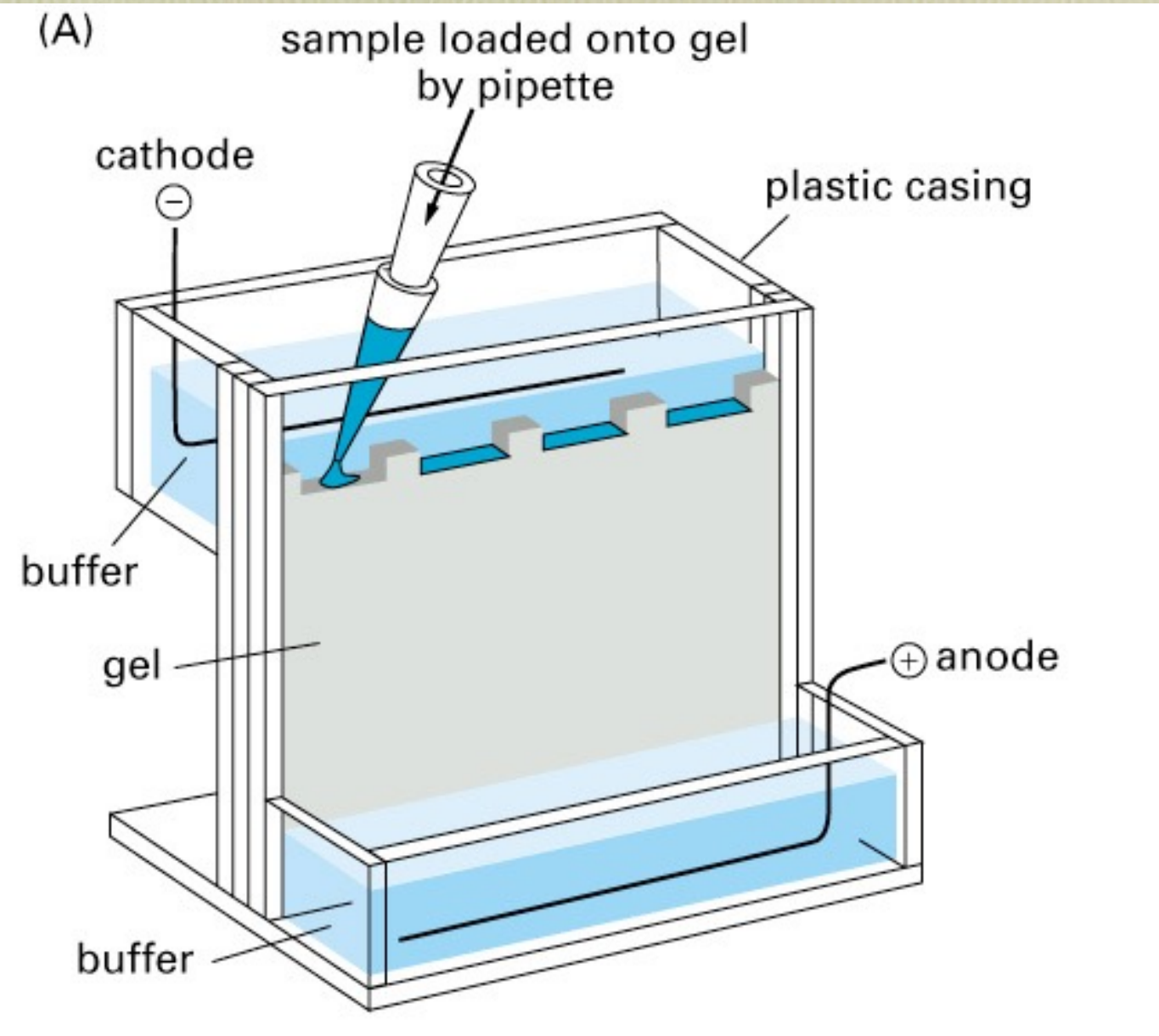
The PCR cycle



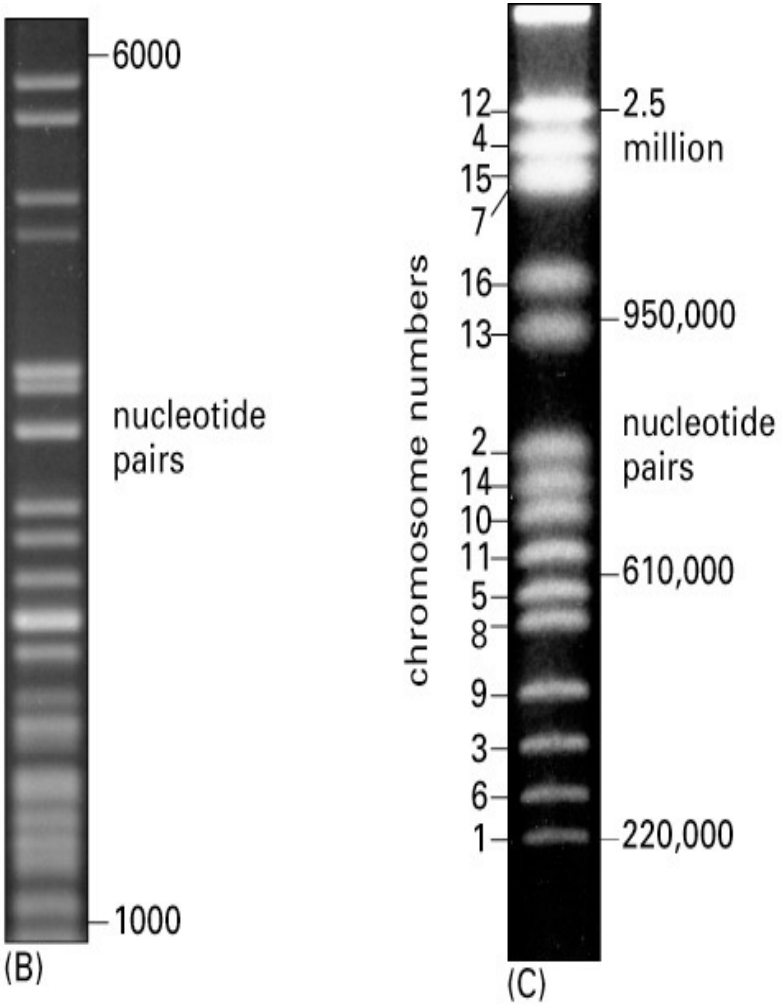
Continued rounds of amplification swiftly produce large number of identical fragments. Each fragment contains the DNA region of interest.

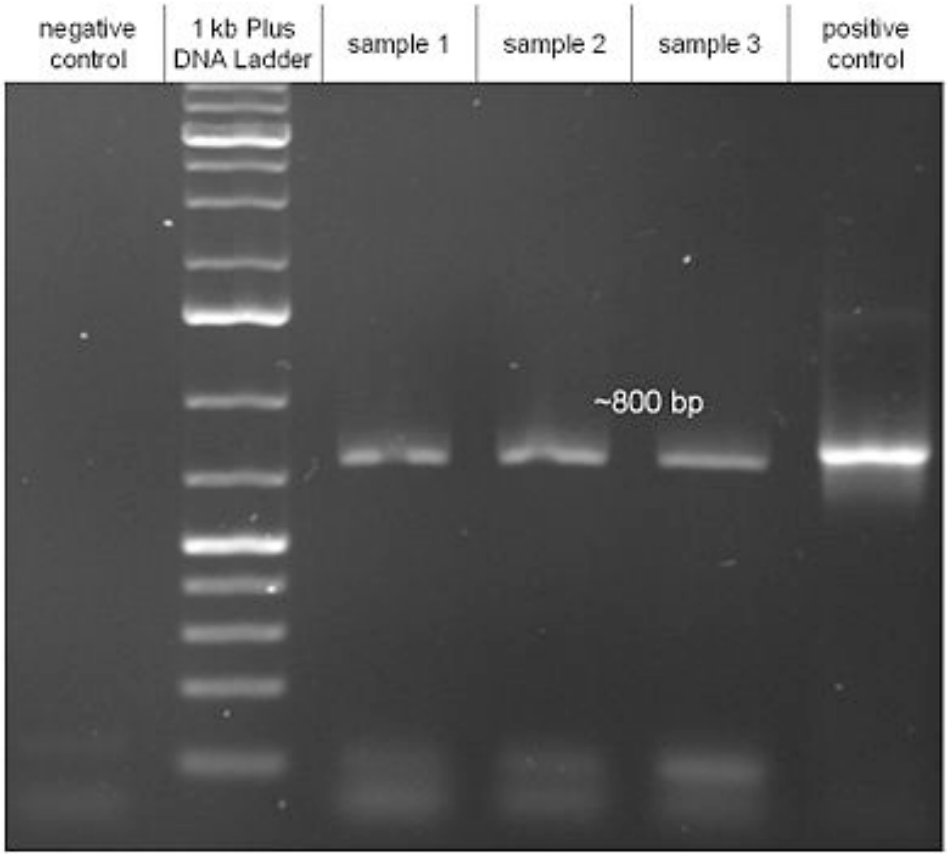



Polymerase Chain Reaction (PCR)
DNA
DNA segment of interest
DNA sample
Sumanas, Inc.


(A)
sample loaded onto gel
by pipette
cathode
plastic casing
buffer
gel
anode
buffer

Gel Electrophoresis Separates DNA Molecules of Different Sizes



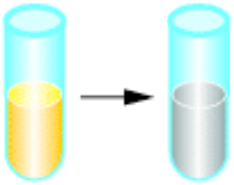


The story of the human gene defective in alkaptonuria

HGO: homogentisate 1,2-dioxygenase

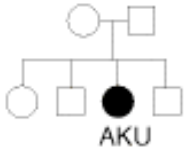
1. Black urine disease

1898



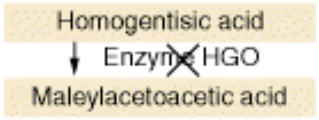
2. Mendelian recessive

1902



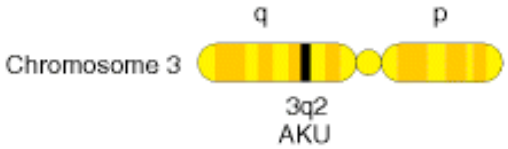
3. Proposed enzyme deficiency

1908



4. AKU gene mapped

1992



5. HGO gene isolated from fungus *Aspergillus*

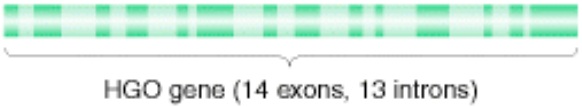
1995

6. *Aspergillus* HGO finds human HGO cDNA

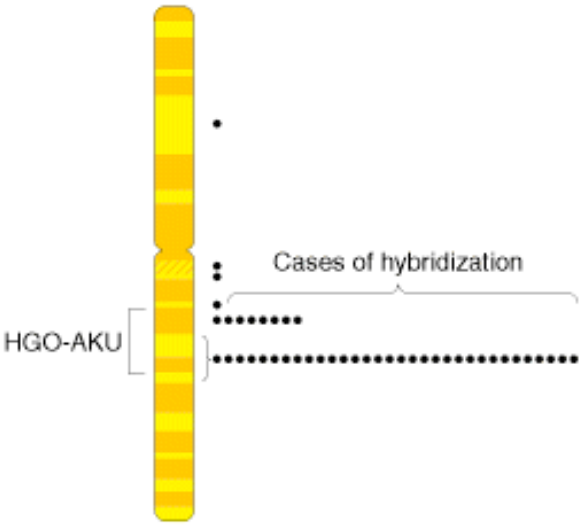
1996

7. HGO as probe finds mRNA in liver

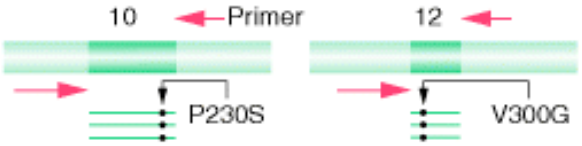
8. cDNA finds gene in lambda genomic library



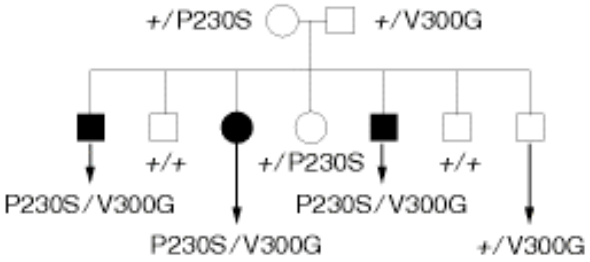
9. HGO clone hybridizes to 3q2



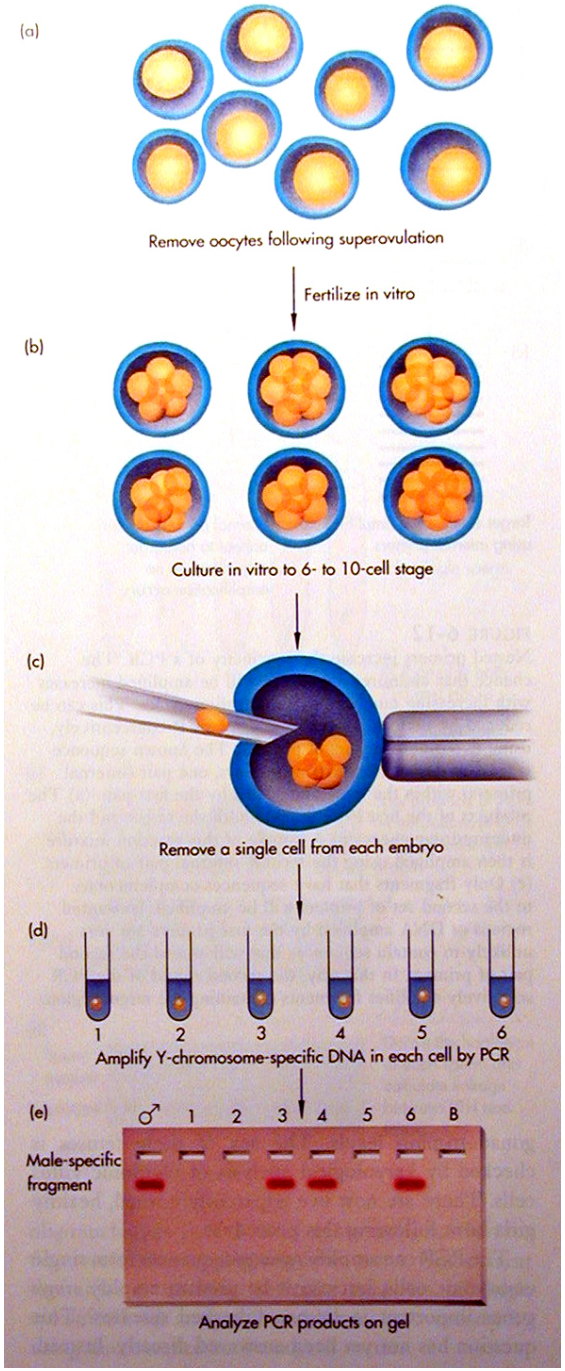
10. PCR of exons 10 and 12 finds mutant sites

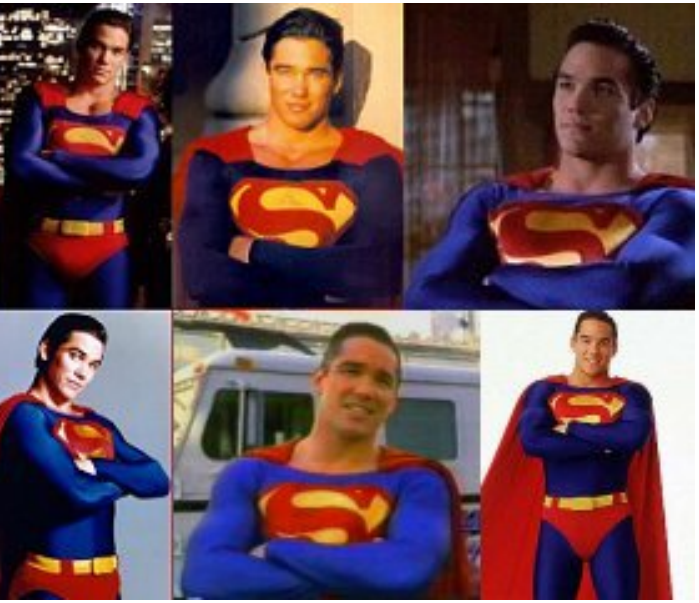


11. Inheritance of mutations



Embryo selection





107850
ARM FOLDING PREFERENCE

Links

TEXT

If in folding his arms the right arm is on top, the person is classed R. Hand clasping (139800) is a comparable trait. Falk and Ayala (1971) concluded that, although both traits are heritable to a significant extent, a simple mendelian hypothesis is not tenable. Ferronato et al. (1974) found no significant correlation between parents and children for arm folding preference, i.e., right arm or left arm on top.

REFERENCES

1. Falk, C. T.; Ayala, F. J. :
Genetic aspects of arm folding and hand clasping. *Jpn. J. Hum. Genet.* 15: 241-247, 1971.

2. Ferronato, S.; Thomas, D.; Sadava, D. :
Preferences for handedness, arm folding, and hand clasping in families. *Hum. Hered.* 24: 345-351, 1974.
PubMed ID : 4461659

CREATION DATE

Victor A. McKusick : 6/4/1986

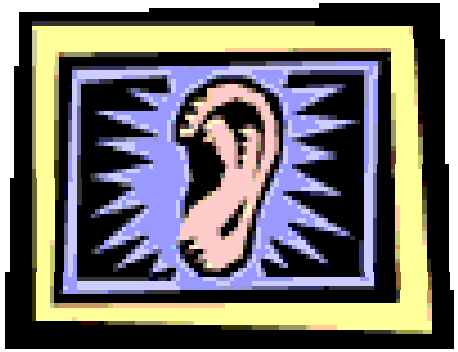
EDIT HISTORY

mimadm : 4/9/1994
supermim : 3/16/1992
supermim : 3/20/1990
ddp : 10/26/1989
marie : 3/25/1988
root : 1/12/1988

129100

Links

EARS, ABILITY TO MOVE



TEXT

Linder (1949) found a frequency of the trait among parents and sibs of probands, leading to the idea that the ability is inherited as a somewhat irregular dominant. In 5 of 24 cases both parents lacked the trait. In Barcelona, Hernandez (1980) found that 19.9% of men and 9.57% of women could move their ears. In males, there was an association with tongue rolling (189300).

REFERENCES

1. Hernandez, M. :
La movilidad del pabellon auditivo. *Trab. Antropol.* XVIII(4): 199-203, 1980.

2. Linder, L. :
The ability to move the ears. *Hereditas* 35 (suppl.): 620-621, 1949.

CREATION DATE

Victor A. McKusick : 6/4/1986

EDIT HISTORY

mimadm : 6/25/1994
supermim : 3/16/1992
supermim : 3/20/1990
ddp : 10/26/1989
marie : 3/25/1988
reenie : 6/4/1986

Mapping genes for human personality

C. Robert Cloninger[1], Rolf Adolfsson[2] & Nenad M. Svrakic[1]

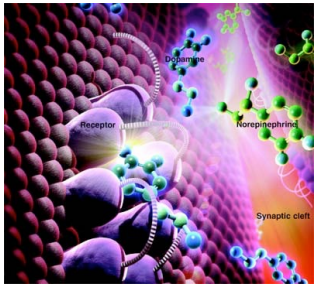
Nature Genetics 12, 3-4 (1996)

Temperament: dynamic organization of the psychobiological systems that regulate automatic responses to emotional stimuli

Four temperament domains:
- Novelty seeking
- Harm avoidance
- Reward dependence
- Persistence

e.g.: **extravert with mature creative character:**
- HIGH Novelty seeking
- LOW Harm avoidance (optimistic)
- HIGH Reward dependence (sociable)
- LOW Persistence

e.g.: **antisocial alcoholics:**
- LOW Novelty seeking
- ~ Harm avoidance (optimistic)
- LOW Reward dependence (sociable)
- ~ Persistence



10% of variation in Novelty seeking is accounted for by a polymorphism of the D4 dopamin receptor gene (D4DR)

Long alleles: HIGH Novelty seeking
(exploratory, thrill seeking, excitable)

Short alleles: LOW Novelty seeking
(deliberate, rigid, orderly)

Ebstein et al. Nature Genetics 12, 78-80 (1996)
Benjamin et al. Nature Genetics 12, 81-84 (1996)

FAITH AGAINST THE ODDS: Worship in the Shadow of Religious Persecution

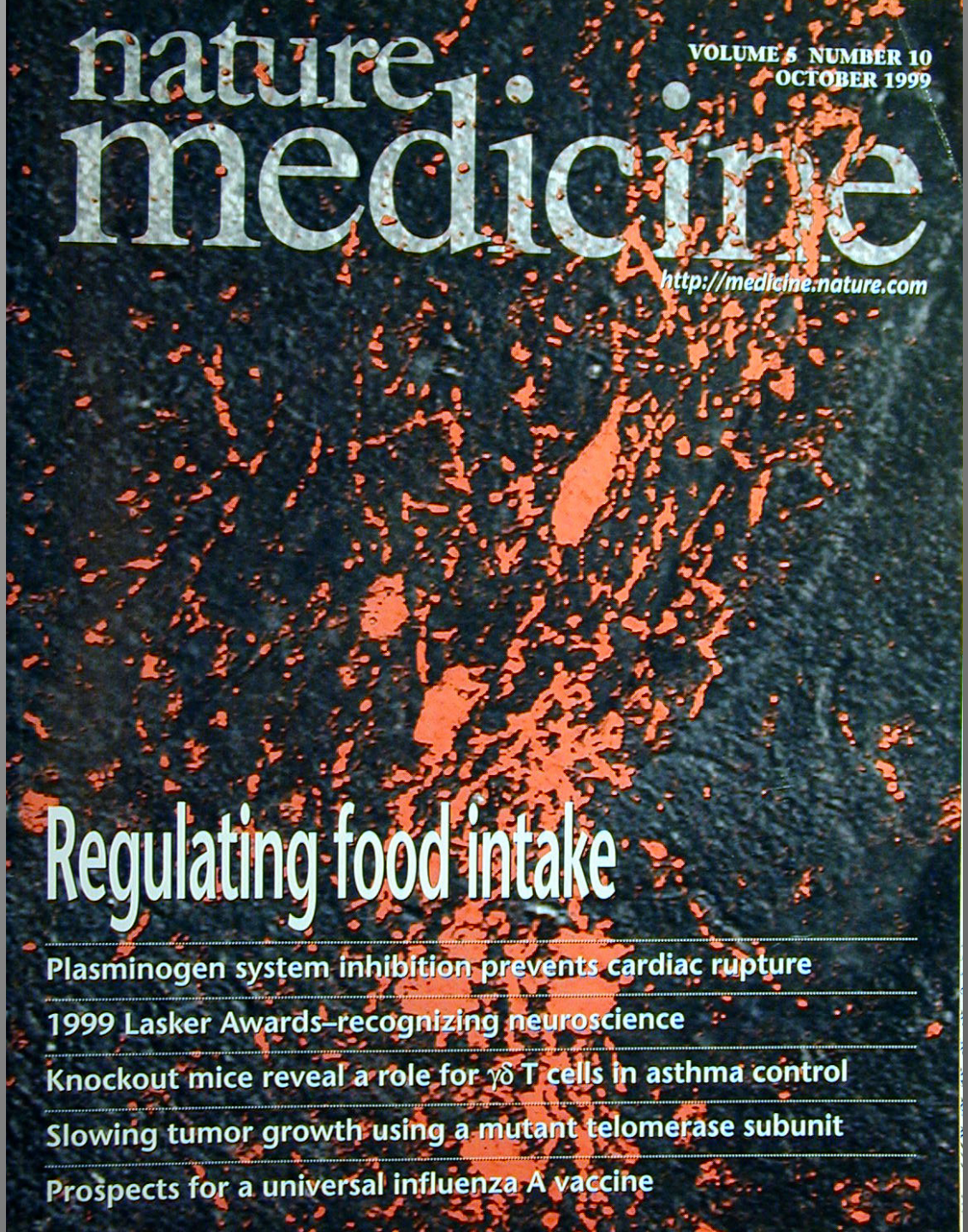
LIFE

WERE YOU BORN THAT WAY?

Personality, temperament, even life choices. New studies show it's mostly in your genes.

APRIL 1998/$3.95

nature medicine
VOLUME 5 NUMBER 10
OCTOBER 1999
http://medicine.nature.com



Regulating food intake

Plasminogen system inhibition prevents cardiac rupture

1999 Lasker Awards–recognizing neuroscience

Knockout mice reveal a role for γδ T cells in asthma control

Slowing tumor growth using a mutant telomerase subunit

Prospects for a universal influenza A vaccine

SEPTEMBER 6, 1999

www.time.com

TIME

TURKEY'S AFTERSHOCK
EAST TIMOR TERROR

WHY WE TAKE RISKS

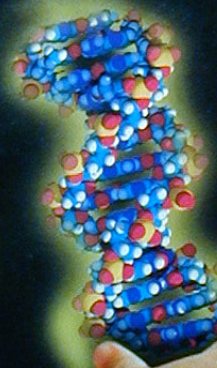
From extreme sports to unprotected sex, thrill seeking is becoming more popular. Here's what makes us go for it.

9 770928 843003 35

SEPTEMBER 13, 1999

TIME

DIANA CRASH VERDICT



What scientists have uncovered about **HOW MEMORY WORKS** and how to improve it

THE I.Q. GENE?

www.time.com

TAMPA, FLORIDA—About 1200 researchers attended the 73rd Annual Meeting of the American Association of Physical Anthropologists here from 14 to 17 April to hear talks on primate genes, behavior, and fossils.

Tracking the Evolutionary History of a "Warrior" Gene

For males, a bit of aggression and risk-taking can earn rewards—just ask real-estate magnate Donald Trump. But inappropriate aggression can lead to violence, addiction, early death, and, the worst fate of all in evolutionary terms, no offspring.

Now, researchers have found signs of this balancing act in the genes of our primate cousins. At the meeting, a team of geneticists traced one genetic variant, an allele that predisposes men to aggressive, impulsive, and even violent behavior, to chimpanzees, gorillas, and other primates. They conclude that this and similar variants arose at least 25 million years ago in a monkey ancestor.

Mad macaque. A genetic variant linked to violence in men has counterparts in primates and can make macaques like this one more aggressive.

In order to be retained for so long, these variants must have conferred some selective advantage on the monkeys—and humans—who carried them, says author Tim Newman, a biological anthropologist at the National Institute on Alcohol Abuse and Alcoholism (NIAAA) in Rockville, Maryland. What we see today as dangerously inappropriate behavior could be "simply out of context," says Newman. "Bold, aggressive males might have been quicker to catch prey or detect threats." Others agree: "If this [allele] has been around that long, then it must be maintained by balancing selection," says biological anthropologist Henry Harpending of the University of Utah in Salt Lake City.

The gene, found on the X chromosome, codes for an enzyme called monoamine oxidase A (MAOA), which breaks down several neurotransmitters in the brain, such as dopamine and serotonin, thus preventing excess neurotransmitters from interfering with communication among neurons. But the gene is polymorphic: A repeat sequence of 30 base pairs has been inserted from three to five times into the promoter region. Fewer repeats mean that less MAOA enzyme is produced and fewer neurotransmitters are removed.

The MAOA gene's effects have been linked to aggression. Lab mice that lack the enzyme are more aggressive, and one human family whose members do not produce the enzyme at all has been linked with violent behavior (*Science*, 18 June 1993, p. 1722). Men who carry the short allele, and so presumably produce a limited amount of enzyme, have been shown to be more likely to be aggressive, impulsive, and even violent if they were abused as children or drink alcohol. Men who had the short variant and were mistreated as boys were four times more likely than other men to have committed violent crimes such as rape, robbery, and assault, according to one study that tracked boys from birth in New Zealand (*Science,* 2 August 2002, p. 851). (Women also inherit the allele, but the effects are easier to study in men, who have only one X chromosome.)

These findings intrigued psychiatrist Klaus-Peter Lesch of the University of Würzburg in Germany, who works with the NIAAA group. His team first found, in macaques, a similar 18-base-pair repeat that also modulates MAOA enzyme activity. And macaques with less enzyme were more aggressive than other macaques when competing for food, says Lesch.

Newman then sampled all apes and many monkeys—almost 600 primates in all—and found the same 30-base-pair repeat seen in humans or the shorter 18-base-pair repeat, among other forms. He noted that apes and Old World (Asian and African) monkeys carried these alleles, whereas New World (South American) monkeys did not. That suggests that the allele arose after New World and Old World monkeys split, but before apes and Old World monkeys diverged about 25 million years ago.

During those 25 million years, aggressive and risk-taking behavior must have had reproductive payoffs for some males, says Newman. But the gene didn't sweep through populations, because if a male was too violent, he probably died before reproducing. Newman suggests that the MAOA gene may offer a rare example of so-called balancing selection, in which selection favors two or more forms of a gene and maintains all the forms in a population. "The human social environment required the development of all kinds of emotional and cognitive capabilities, and [it] demanded variation in impulsivity in humans," agrees David Goldman, a member of the NIAAA team. "It's what I call the warrior vs. the worrier." In other words, primate politics has long favored more than one route to success.

Chimpanzee Gang Warfare

Primatologists have long known that chimpanzees can be demonic: Bands of males routinely head to the borders of their territory to seek, and sometimes destroy, foreign chimpanzees. But what triggers these patrols, and why do males of the troop—who compete fiercely with one another most of the time—seem to cooperate while on patrol? The answer, it seems, may be a mob mentality.

In a study of a group of 150 chimpanzees at Ngogo in the Kibale National Park in Uganda, researchers found that chimpanzees went on patrol only after they had assembled enough members to have overwhelming force. Patrols require "safety in numbers" because attacking a foreign chimpanzee is dangerous, explains primatologist John Mitani of the University of Michigan, Ann Arbor, co-author of the study with primatologist David Watts of Yale University.

Once a patrol formed, its members exhibited frequent displays of male bonding. "Cooperation among males is rare among animals," says Watts. "It is conspicuous that closely related chimpanzees and humans deindividualize to engage in this coalitional aggression against outsiders."

CREDIT: DEE HIGLEY

Genetic variation in the vasopressin receptor 1a gene (*AVPR1A*) associates with pair-bonding behavior in humans

Hasse Walum*†‡, Lars Westberg†§, Susanne Henningsson§, Jenae M. Neiderhiser¶, David Reiss‖, Wilmar Igl*, Jody M. Ganiban, Erica L. Spotts††, Nancy L. Pedersen*, Elias Eriksson§, and Paul Lichtenstein***

*Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Box 281, S-171 77 Stockholm, Sweden; §Department of Pharmacology, Institute of Neuroscience and Physiology, University of Gothenburg, Box 431, S 405 30 Gothenburg, Sweden; ¶Department of Psychology, Pennsylvania State University, University Park, PA 16802; ‖Yale Child Study Center, Yale University, New Haven, CT 06520; **Department of Psychology, The George Washington University, Building GG 2125 G St NW, Washington, DC 20052; and ††Behavioral and Social Research Program, National Institute on Aging, Bethesda, MD 20892-9205

Edited by Solomon H. Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved July 14, 2008 (received for review March 28, 2008)

RS3 allele "334" present in 2 our 5 men

PARTNER BONDING: Renders men distant and disagreeable rather than emotionally close and available

MARITAL STATUS: Is predictive of men not getting married (32% of the man with two alleles were living to women without getting married vs. 17% of men without any allele)

PERCEIVED MARITAL PROBLEMS: Men with two copies of the allele had twice the risk of experiencing marital dysfunction with a threat of divorce

Table 3. Effect of 0, 1 or 2 334 alleles on male reports on the Partner Bonding Scale, marital crisis, and marital status

	Number of 334 alleles					
Measure	0	1	2	*df*	*F*	*P*
Mean score for the Partner Bonding Scale in the three groups						
Partner Bonding Scale	48.0 (6.50)	46.3 (6.16)	45.5 (6.71)	2, 143	8.40	0.0004
Frequency and column-wise percentage of subjects reporting marital crisis/threat of divorce in the three groups						
Have you experienced marital crisis or threat of divorce during the last year?						
No	469 (85%)	277 (84%)	27 (66%)	2, 143	5.00	0.008
Yes	81 (15%)	51 (16%)	14 (34%)			
Frequency and column-wise percentage of subjects being married or cohabiting in the three groups						
Marital status						
Married	457 (83%)	275 (84%)	28 (68%)	2, 143	4.36	0.01
Cohabiting	96 (17%)	52 (16%)	13 (32%)			

Values for the Partner Bonding Scale are means with standard deviation in brackets.

SMITTEN
DAILY SEX & RELATIONSHIPS BLOG

See all blog posts

Does Your Man Have "The Cheating Gene?"

Thursday, 09/ 4/2008 at 11:02 AM Comments (12)

V1aR gene RS3 allele 334

the "cheating gene"
the "infidelity gene"
the "divorce gene"

the "committment gene"
the "bonding gene"
the "monogamy gene"

My grandfather just wasn't "the cheating type." Although he was a tall, dark and handsome fighter pilot in WWII, he wouldn't have cheated on my grandmother in a million years. I figured he was just a great guy. But new research shows that his good behavior might have been in his genes. And other men, because of their genes, may cheat more often!

Swedish scientists have just made a shocking discovery: Two out of every five men (that's a lot!) carry a gene variant, known as an allele. Men with one or two of the alleles were found in the study to be more likely to have marital problems and get divorced than other men. Women involved with these men were also more likely describe their partners as distant and disagreeable.

Yikes, that's pretty heavy news. Have you met guys who seem like the "cheating type"? On the flip, have you met guys who seem destined to be faithful? If you're dating someone, would you want to test him for the gene before walking down the aisle? And what if you found out he had it--would that change your mind about marrying him? Tell me your thoughts, ladies!

Genesis BioLabs

Premarital genetic screening usually includes tests for rare genetic disorders.

Genesis Biolabs offers the first genetic screen for marital success!*

Screening for AVPR1a, known alternately as the "ruthlessness" gene or the "bonding" gene, is likely an indicator of marital happiness. Marriages born out of mutual respect and mutual interest rather than self-interest are much more likely to succeed and probably less likely to end in divorce. Is your fiancé just after your money? Those with the "ruthlessness" gene may very well be. Those with the altruistic version of AVPR1a probably aren't. Ruthless people will lie, cheat and steal to get what they want. Genetics may not be a guaranteed indicator of human behavior and motivation [genetics is only one half of the nature vs. nurture debate] but genes don't lie. Before you make a lifetime commitment, have your fiancé tested.

"Ruthlessness/Bonding" Gene Test.

I have read and agree to the Terms of Use

Order a Bonding Gene Test : $99

Buy Now

Authorize.Net Simple Checkout

Please agree to the terms of use!

You will receive 1 mouth swab and collection tube per test, in a return package, along with specific instructions on how to collect the samples. Ask your fiancée, significant other, business partner and/or elected representative to get these genetic tests done as soon as possible. This is for informational purposes only and is not a medical diagnosis. Consult with your doctor.

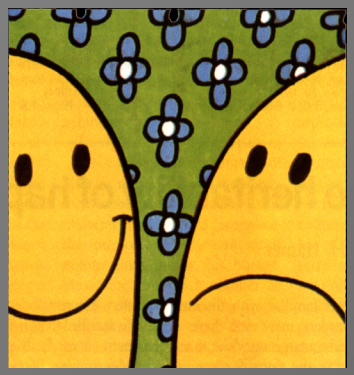
The heritability of happiness

Dean H. Hamer

Nature Genetics 14, 125-126 (1996)

"Hello, said Mr. Happy. "I'm Mr. Happy." "Oh, are you indeed", sniffed the person who looked like Mr. Happy but wasn't. "Well, my name is Mr. Miserable, and I'm the most miserable person in the world." "Why are you so miserable?" asked Mr. Happy. "Because I am." replied Mr. Miserable.

Happy Talk



Longitudinal study of 1,380 pairs of twins born between 1936-1955. Measure of happiness by the Well Being scale of the Multidimensional Personality Questionnaire

Correlation for Well Being scale:

If twins grew up together:
0.44 for monozygotic twins
0.08 for dizygotic twins

If twins were separated at infancy:
0.52 for monozygotic twins
0.02 for dizygotic twins

40-50% of happiness hereditable
0% due to shared environment
including parenting style, socioeconomic status and educational system
50-60% due non shared environment
including unique life experiences

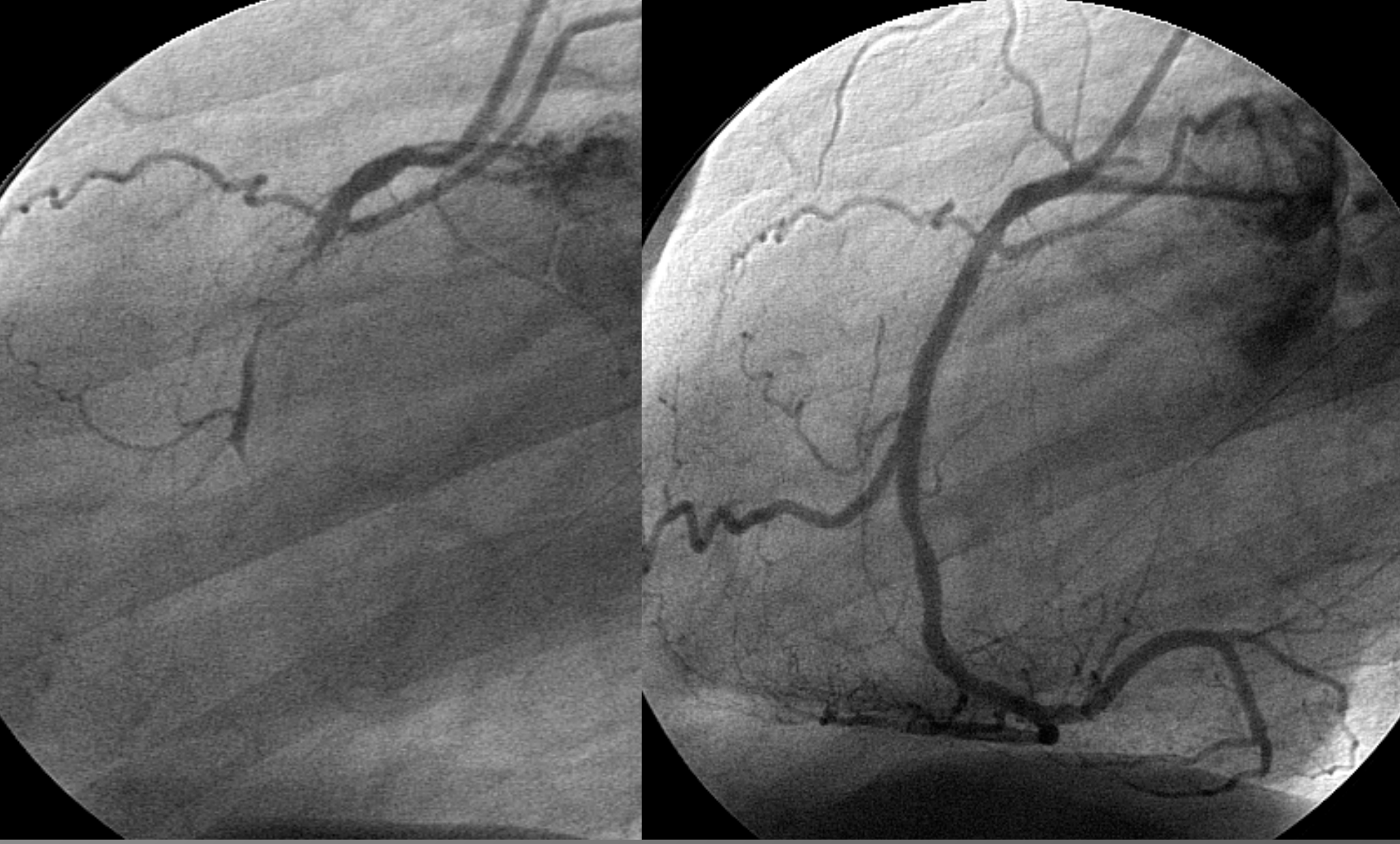
After 5 years:

cross-time correlation: **0.5 for both type of twins**

cross-time
cross-twin correlation: **0.40 for monozygotic twins**
0.07 for dizygotic twins

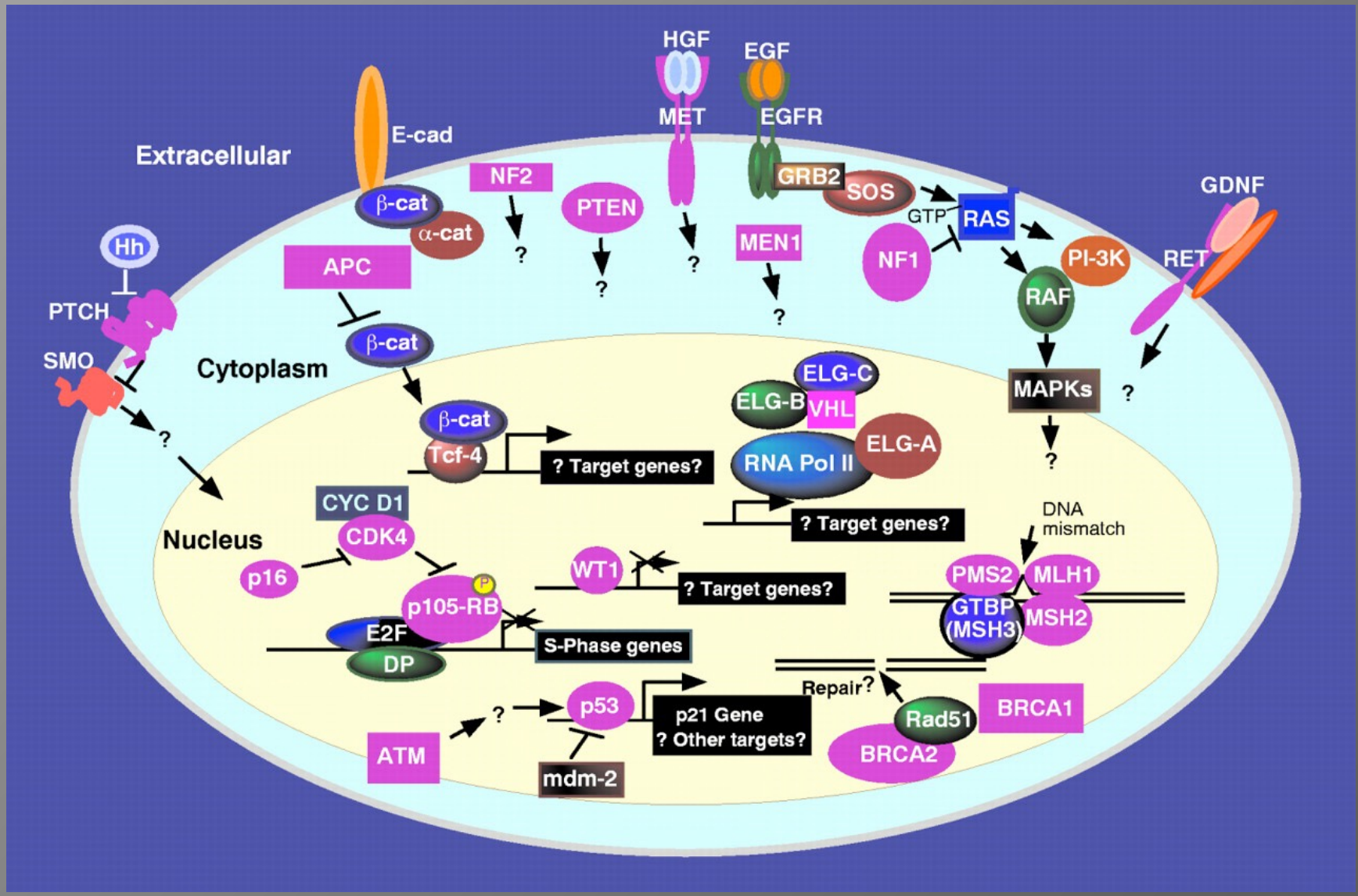
0.4/0.5 = 80% of stable happiness is genetic

Lykken and Tellegen. Psychol. Sci. 7, 186-189 (1996)



L’infarto del miocardio in soggetti giovani (prima dei 40 anni)

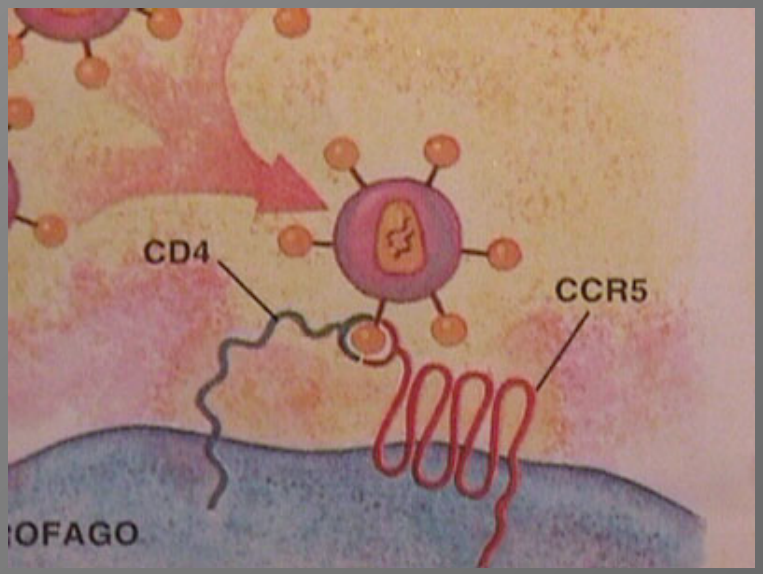
Meccanismi molecolari della crescita tumorale

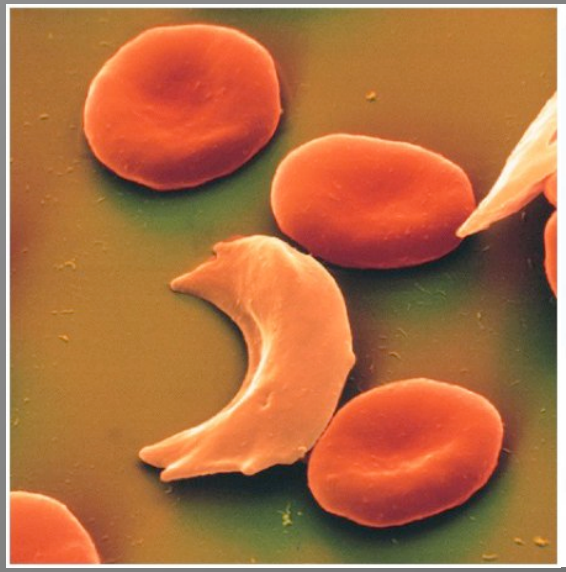


POLIMORFISMI/ MUTAZIONI PROTETTIVI:

CCR5 E HIV

ANEMIA FALCIFORME E MALARIA





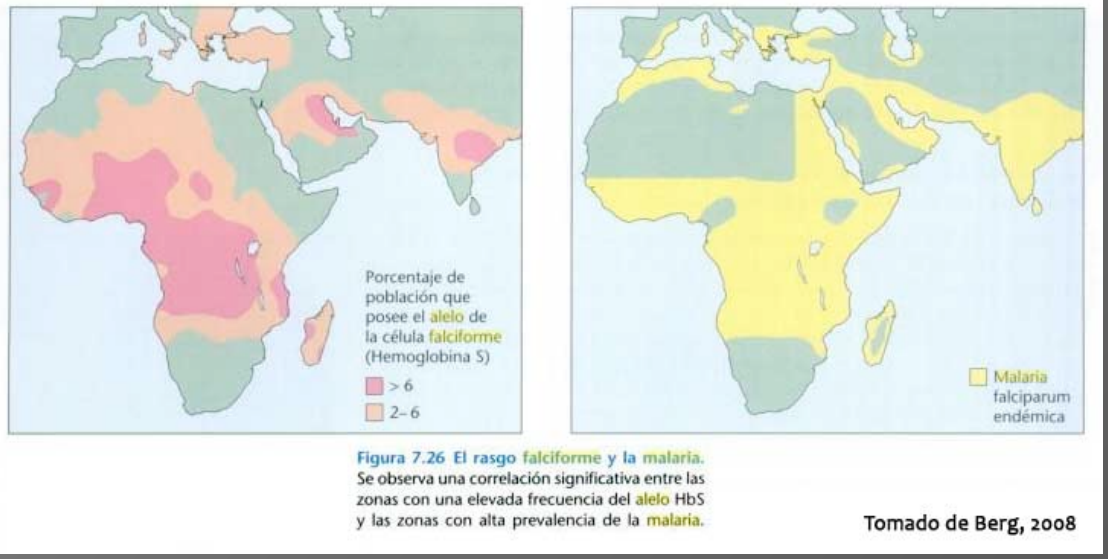


Figura 7.26 El rasgo falciforme y la malaria. Se observa una correlación significativa entre las zonas con una elevada frecuencia del alelo HbS y las zonas con alta prevalencia de la malaria.

Tomado de Berg, 2008

Human Identity Testing

- Forensic cases -- **matching suspect with evidence**
- Paternity testing -- **identifying father**
- Historical investigations
- Missing persons investigations
- Mass disasters -- **putting pieces back together**
- Convicted felon DNA databases

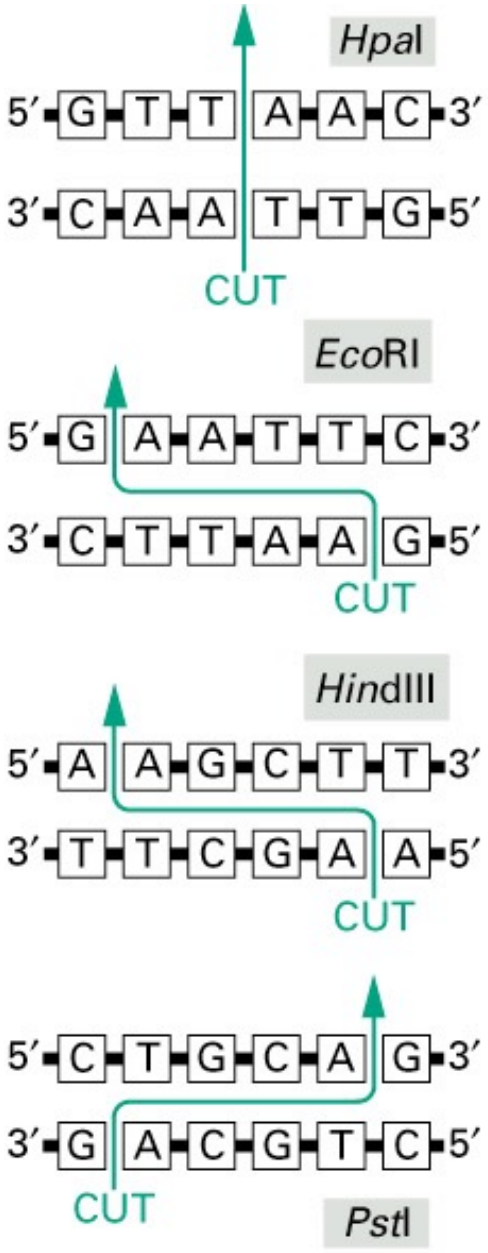
Sources of Biological Evidence

- **Blood**
- **Semen**
- **Saliva**
- **Urine**
- **Hair**
- **Teeth**
- **Bone**
- **Tissue**

Brief History of Forensic DNA Typing

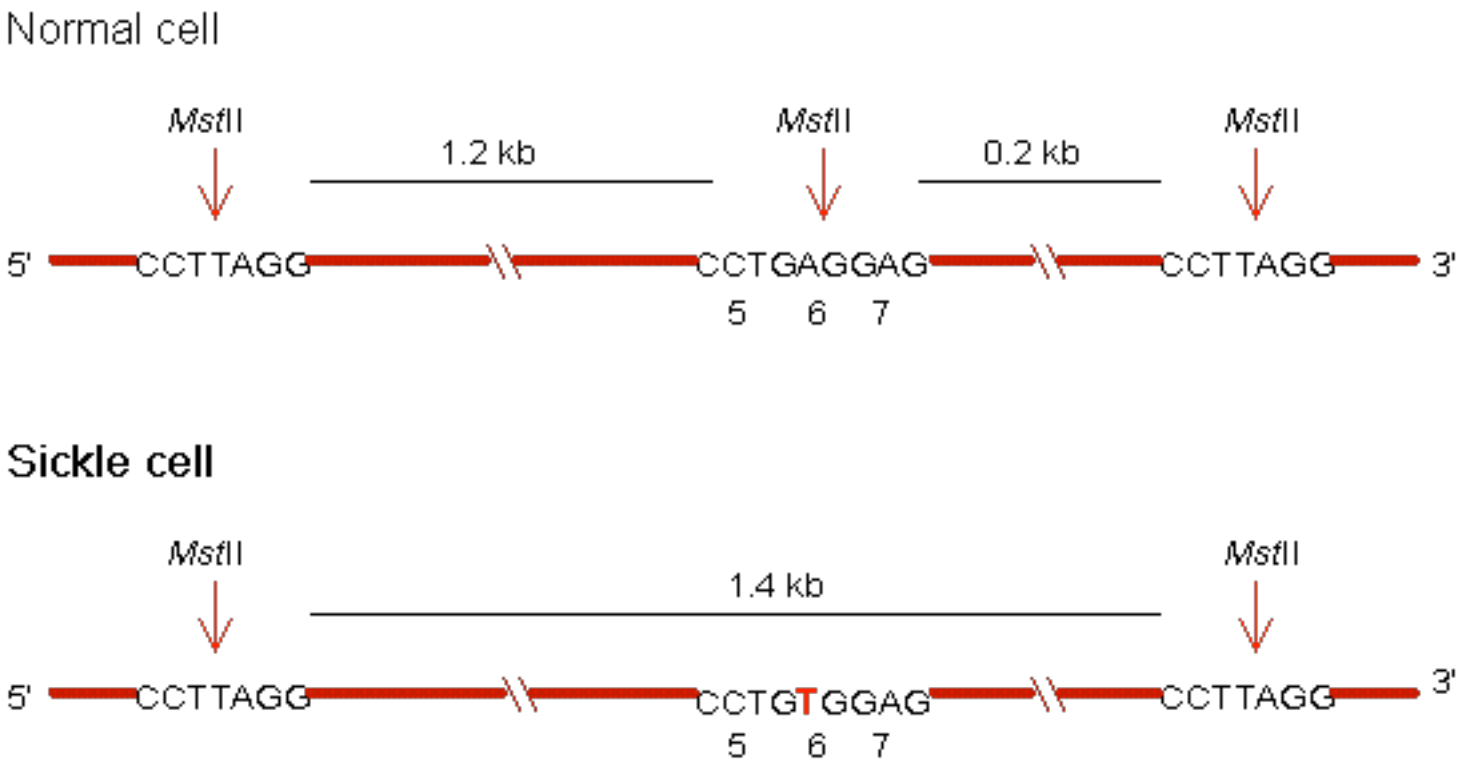
- 1980 - Ray White describes first polymorphic RFLP marker
- 1985 - Alec Jeffreys discovers multilocus VNTR probes
- 1985 - first paper on PCR
- 1988 - FBI starts DNA casework
- 1991 - first STR paper
- 1995 - FSS starts UK DNA database
- 1998 - FBI launches CODIS database

Restriction nucleases



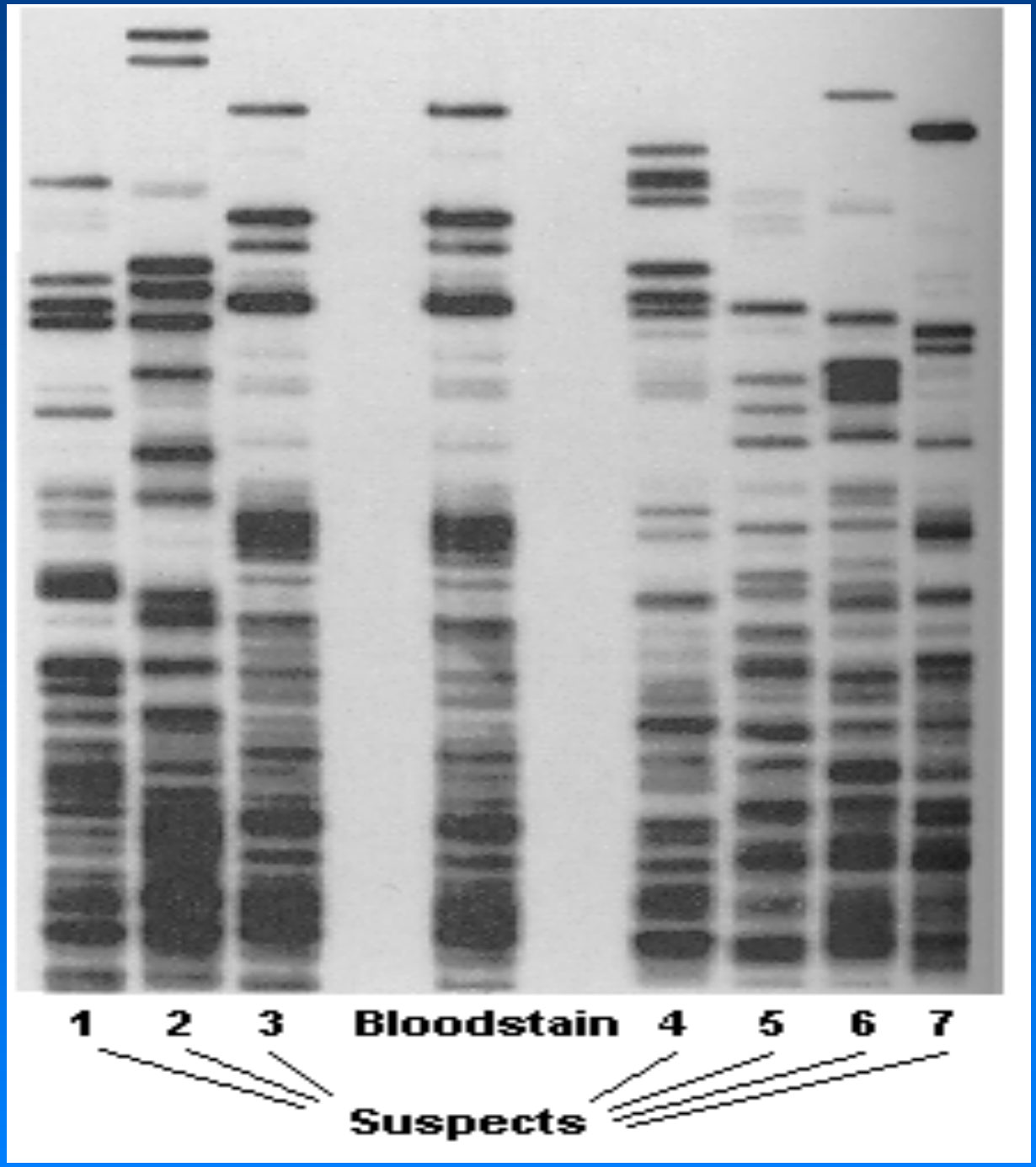
Restriction Fragment Length Polymorphism (RFLP)

Polymorphism refers to the DNA sequence variation between individuals of a species. If the sequence variation occurs at the restriction sites, it could result in RFLP. The most well known example is the RFLP due to b globin gene mutation.

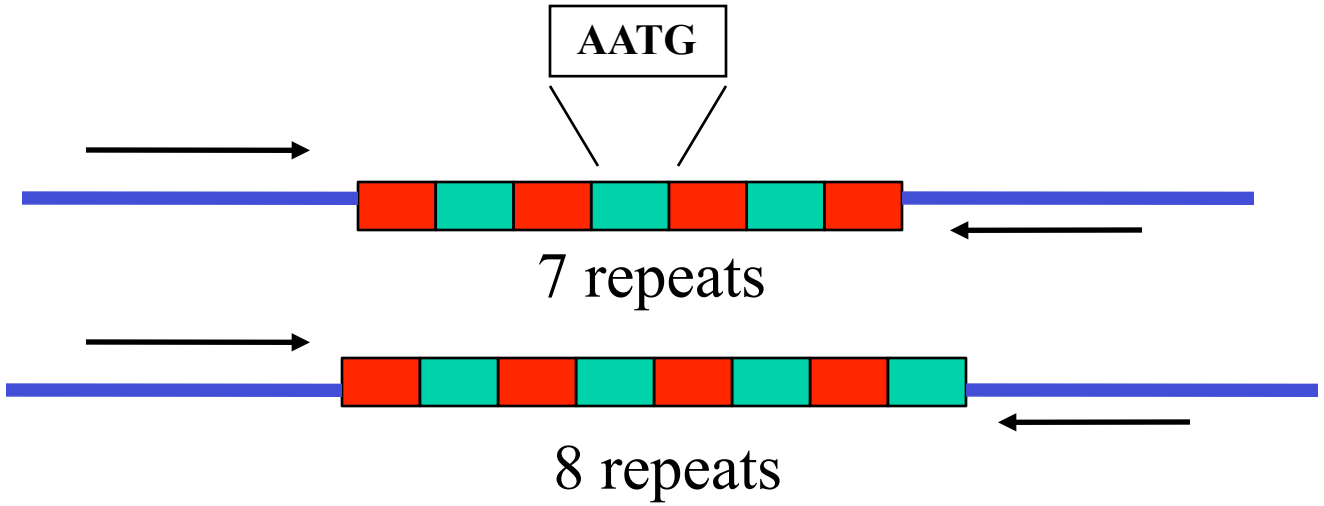


Restriction Fragment Length Polymorphism (RFLP) resulting from b-globin gene mutation. In the normal cell, the sequence corresponding to 5th to 7th amino acids of the b-globin peptide is CCTGAGGAG, which can be recognized by the restriction enzyme *Mst*II. In the sickle cell, one base is mutated from A to T, making the site unrecognizable by *Mst*II. Thus, *Mst*II will generate 0.2 kb and 1.2 kb fragments in the normal cell, but generate 1.4 kb fragment in the sickle cell.

RFLP



Short Tandem Repeats (STRs)

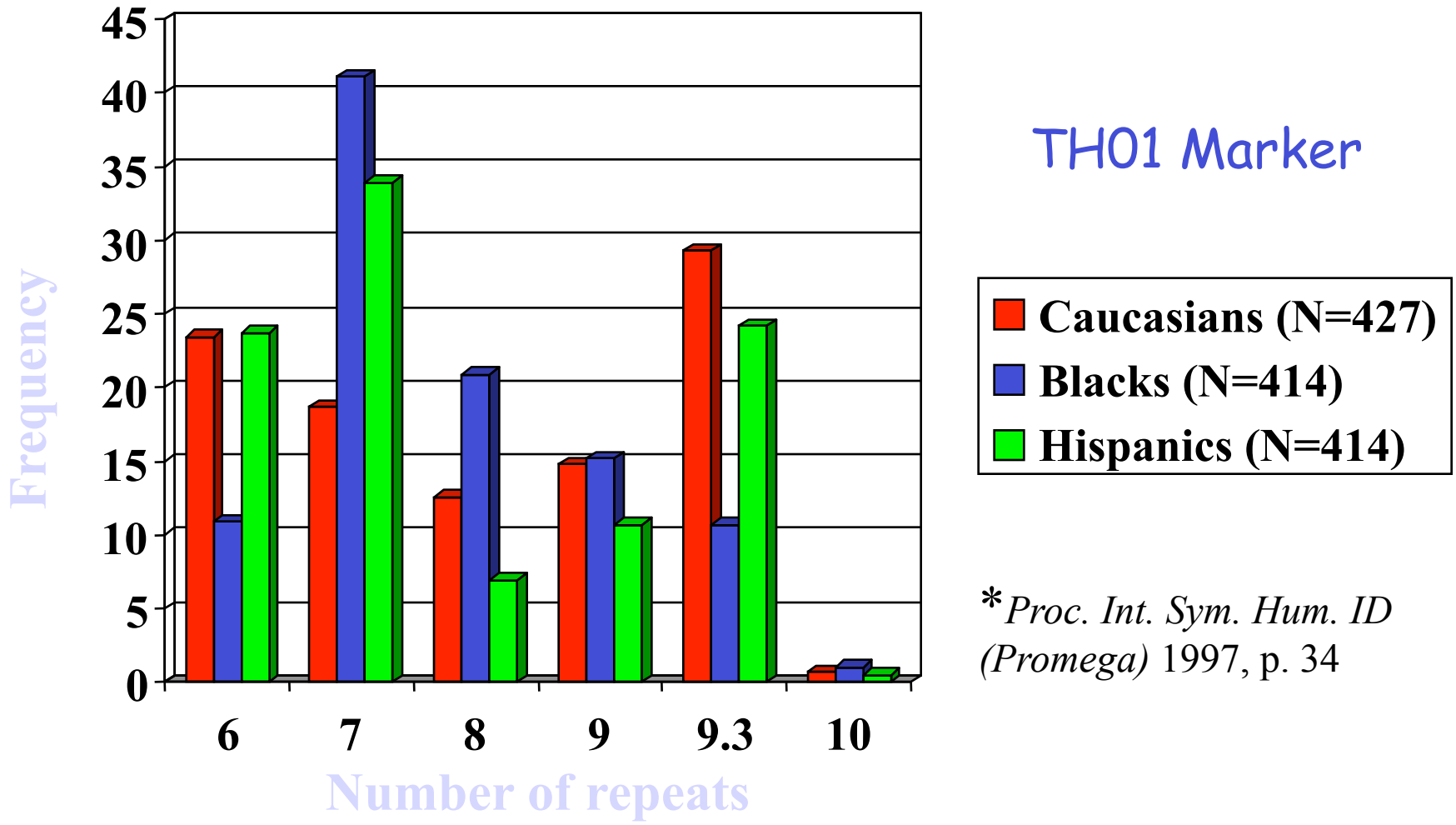


the repeat region is variable between samples while the flanking regions where PCR primers bind are constant

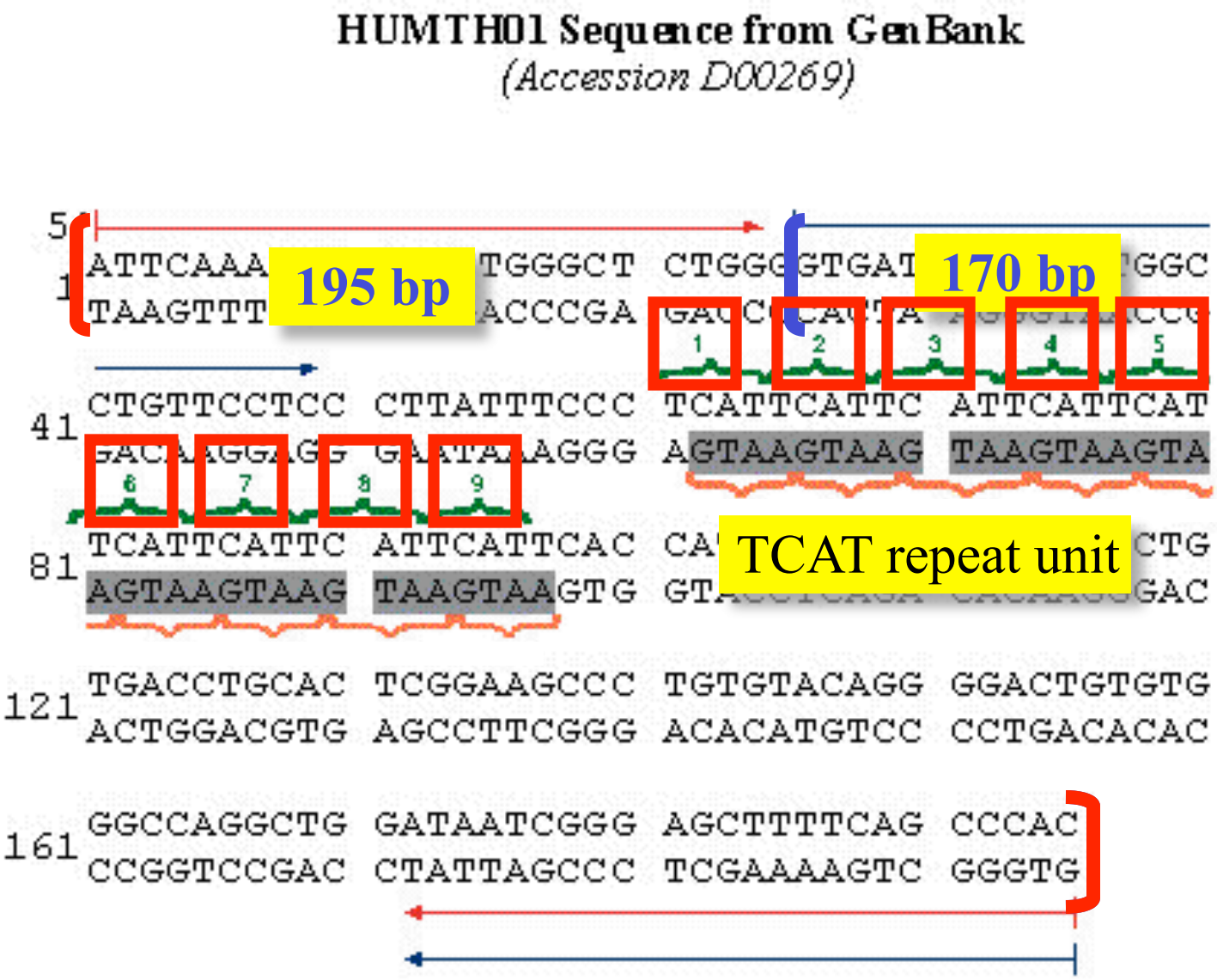
Homozygote = both alleles are the same length

Heterozygote = alleles differ and can be resolved from one another

STR Allele Frequencies

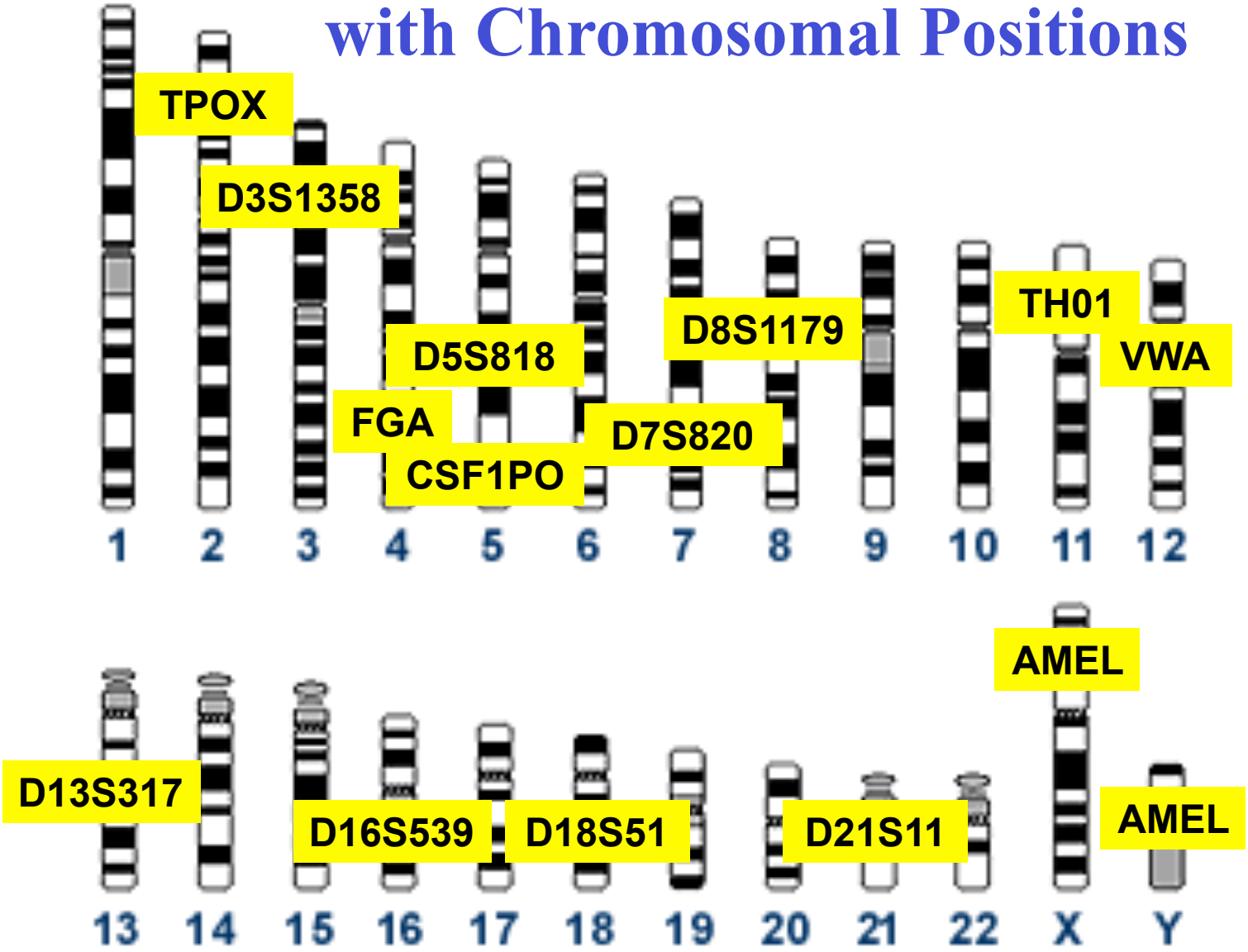


Proc. Int. Sym. Hum. ID (Promega) 1997, p. 34



Different primer sets produce different PCR product sizes for the same STR allele

13 CODIS Core STR Loci with Chromosomal Positions

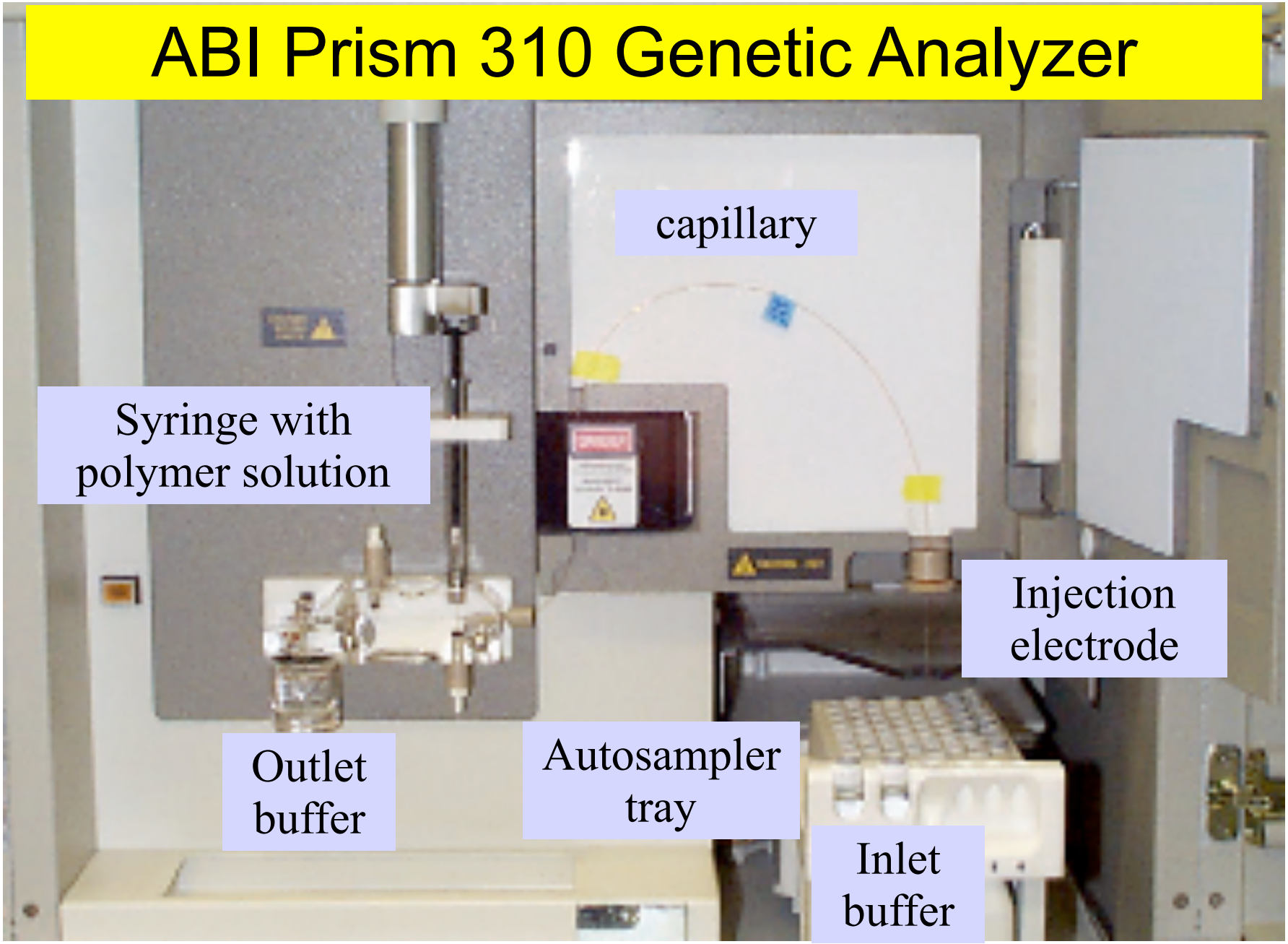


FBI's CODIS DNA Database

Combined **D**NA **I**ndex **S**ystem

- 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

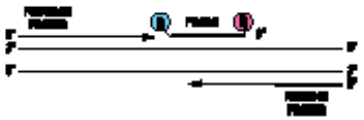
ABI Prism 310 Genetic Analyzer



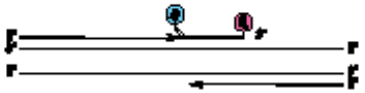
Real Time PCR

Fluorogenic 5' nuclease chemistry

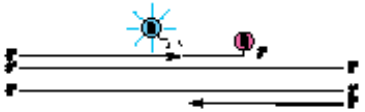
1. *Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' ends respectively, of a TaqMan® probe.*



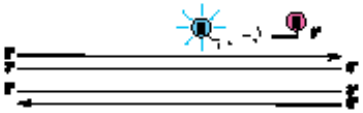
2. *Strand displacement: When the probe is intact, the reporter dye emission is quenched.*



3. *Cleavage: During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe.*



4. *Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence.*

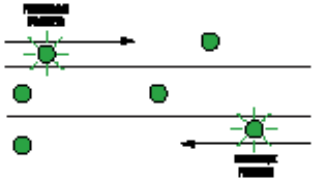


SYBR® Green I dye assay chemistry

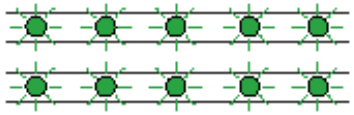
1. *Reaction set-up: The SYBR® Green I dye fluoresces when bound to double-stranded DNA.*

2. *Denaturation: When the DNA is denatured, the SYBR® Green I dye is released and the fluorescence is drastically reduced.*

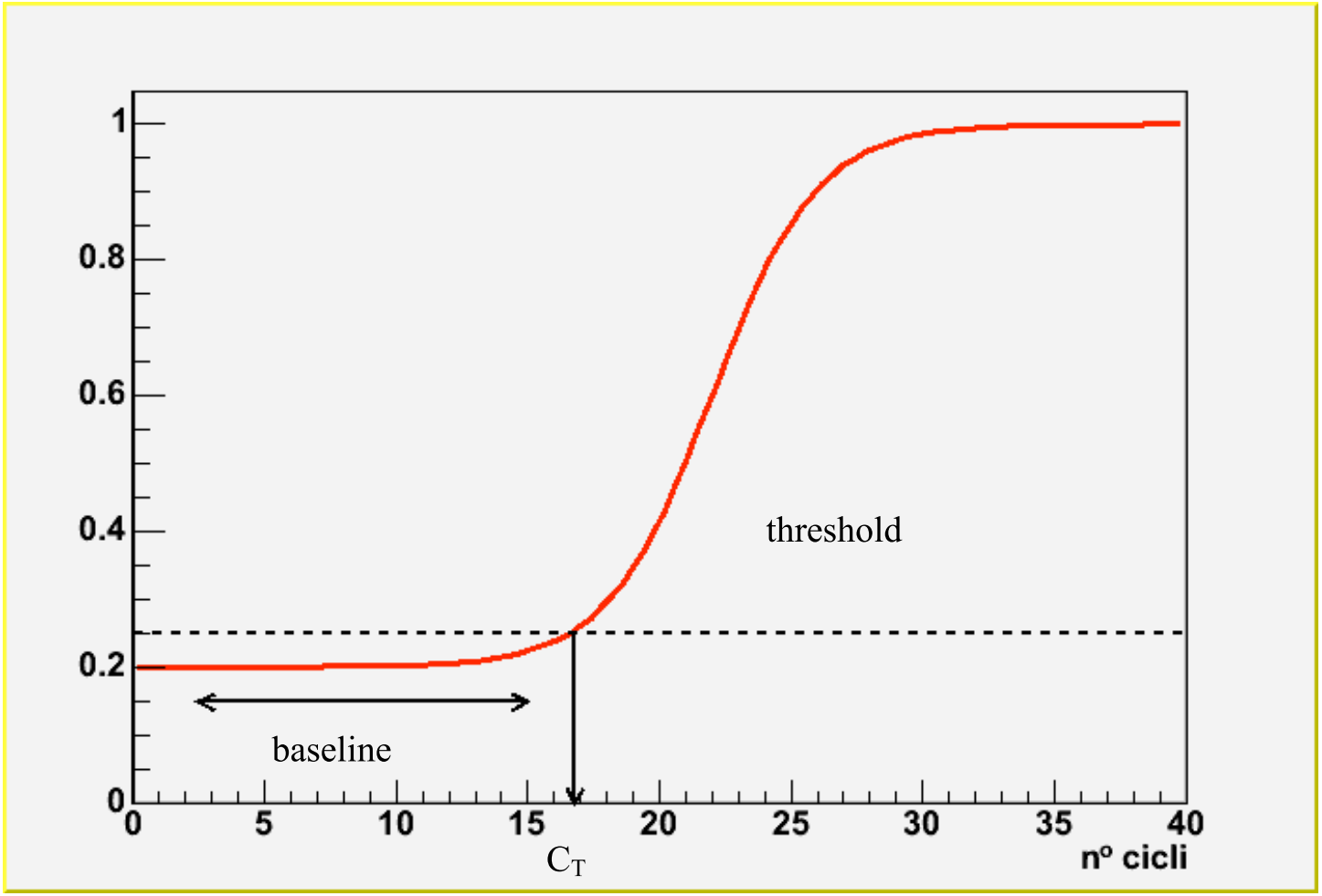
3. *Polymerization: During extension, primers anneal and PCR product is generated.*



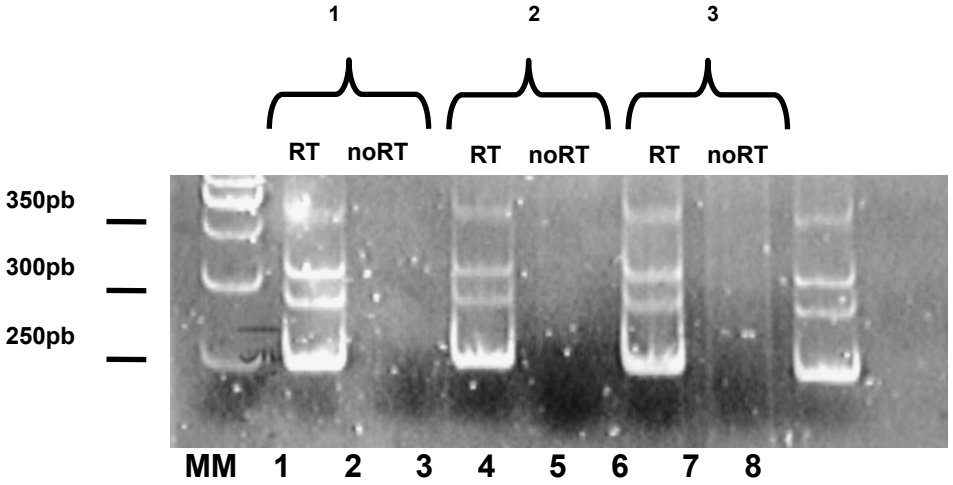
4. *Polymerization complete: SYBR® Green I dye binds to the double-stranded product, resulting in a net increase in fluorescence detected by the 7000 system.*



The amplification plot

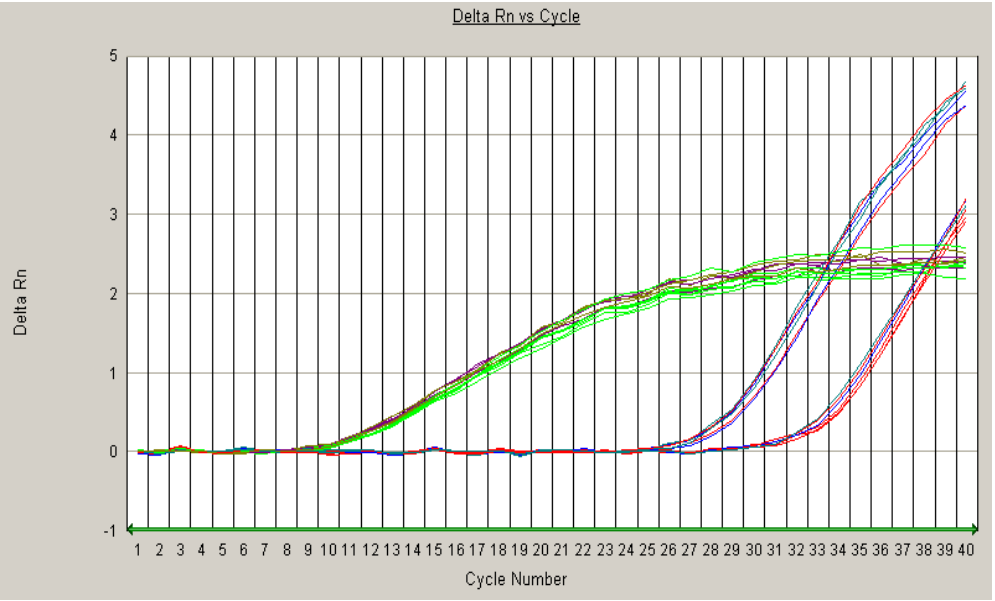


Gene expression analysis



Traditional PCR

Real Time PCR



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

DNA Use in Forensic Cases

- Most are rape cases (>2 out of 3)
- Looking for match between evidence and suspect
- Must compare victim's DNA profile

Challenges

- Mixtures must be resolved
- DNA is often degraded
- Inhibitors to PCR are often present

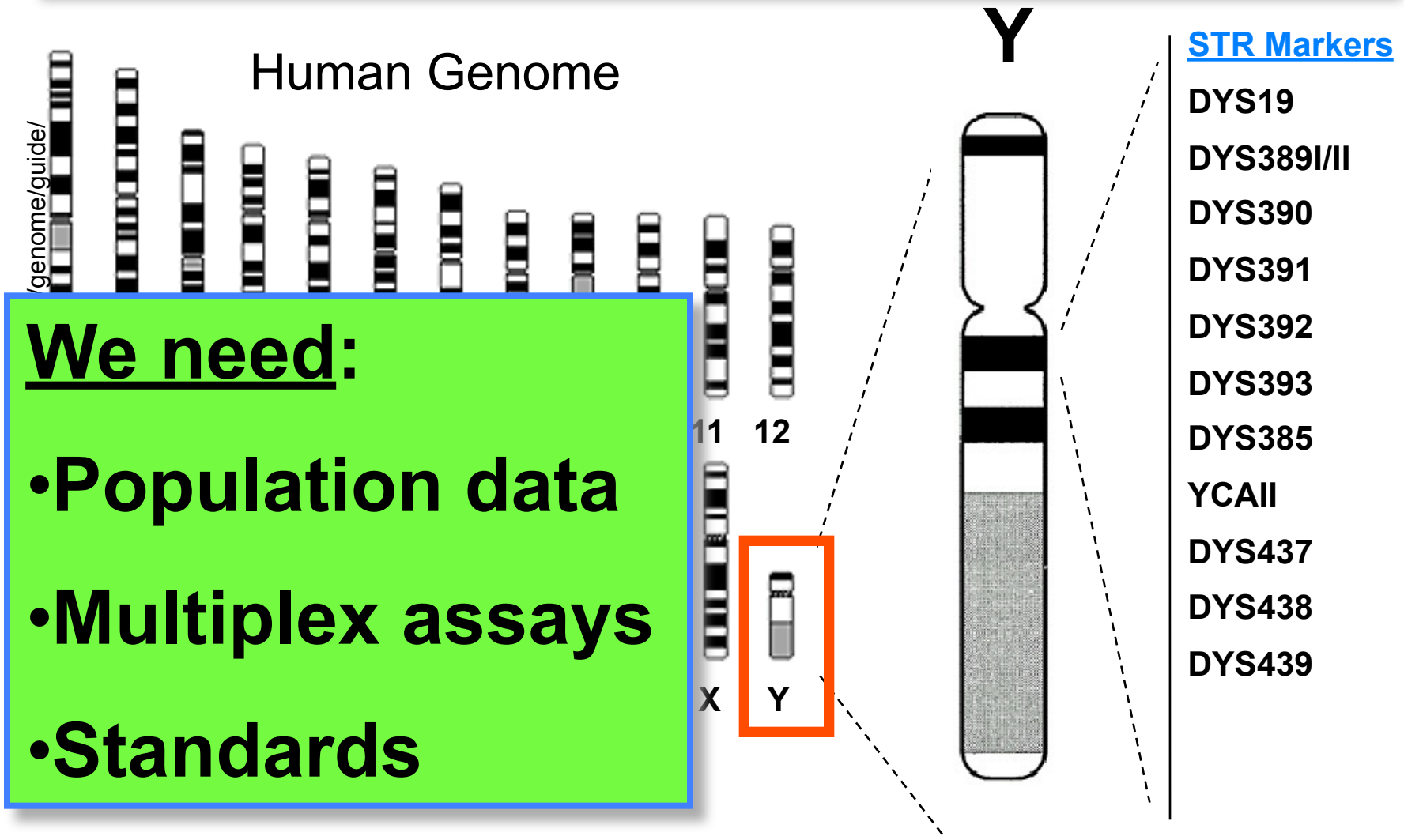
Highly Multiplexed Assays for Measuring Polymorphisms on the Y-Chromosome

International Society of Forensic Genetics

August 30, 2001

John Butler Rich Schoske Pete Vallone

There is a growing interest in the Y-chromosome to aid forensic and paternity testing…
(>50 presentations here at ISFG on Y markers)



We need:

- **Population data**
- **Multiplex assays**
- **Standards**

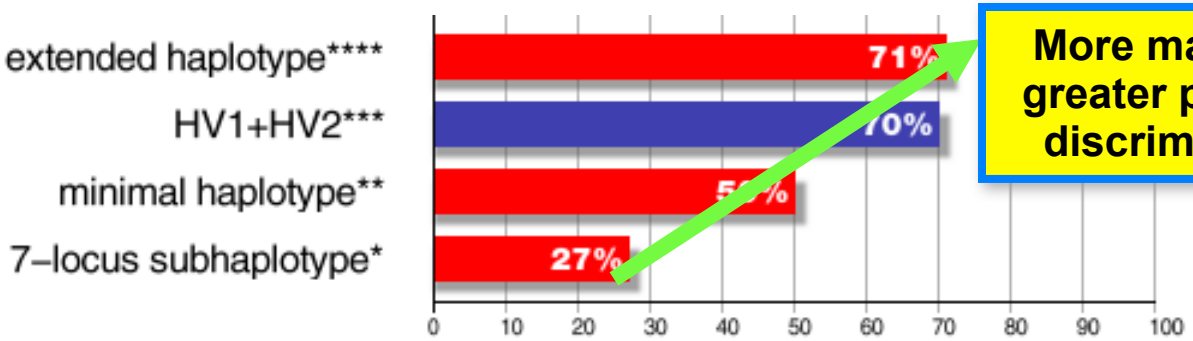
European Y-STR Haplotype Reference Database

Created by Sascha Willuweit and Lutz Roewer
Institute of Legal Medicine, Humboldt-Universität Berlin, Germany
in cooperation with Michael Krawczak (Cardiff), Manfred Kayser (Leipzig) and Peter de Knijff (Leiden)

This database has been accessed **14809** times since 01/01/2000. Last haplotype entry **3/26/2001**

Current state of the database: **45** European population samples
5529 minimal haplotypes, **2196** of these are extended haplotypes

DYS19 DYS389I DYS389II DYS390 DYS391 DYS392 DYS393 DYS385 YCAII

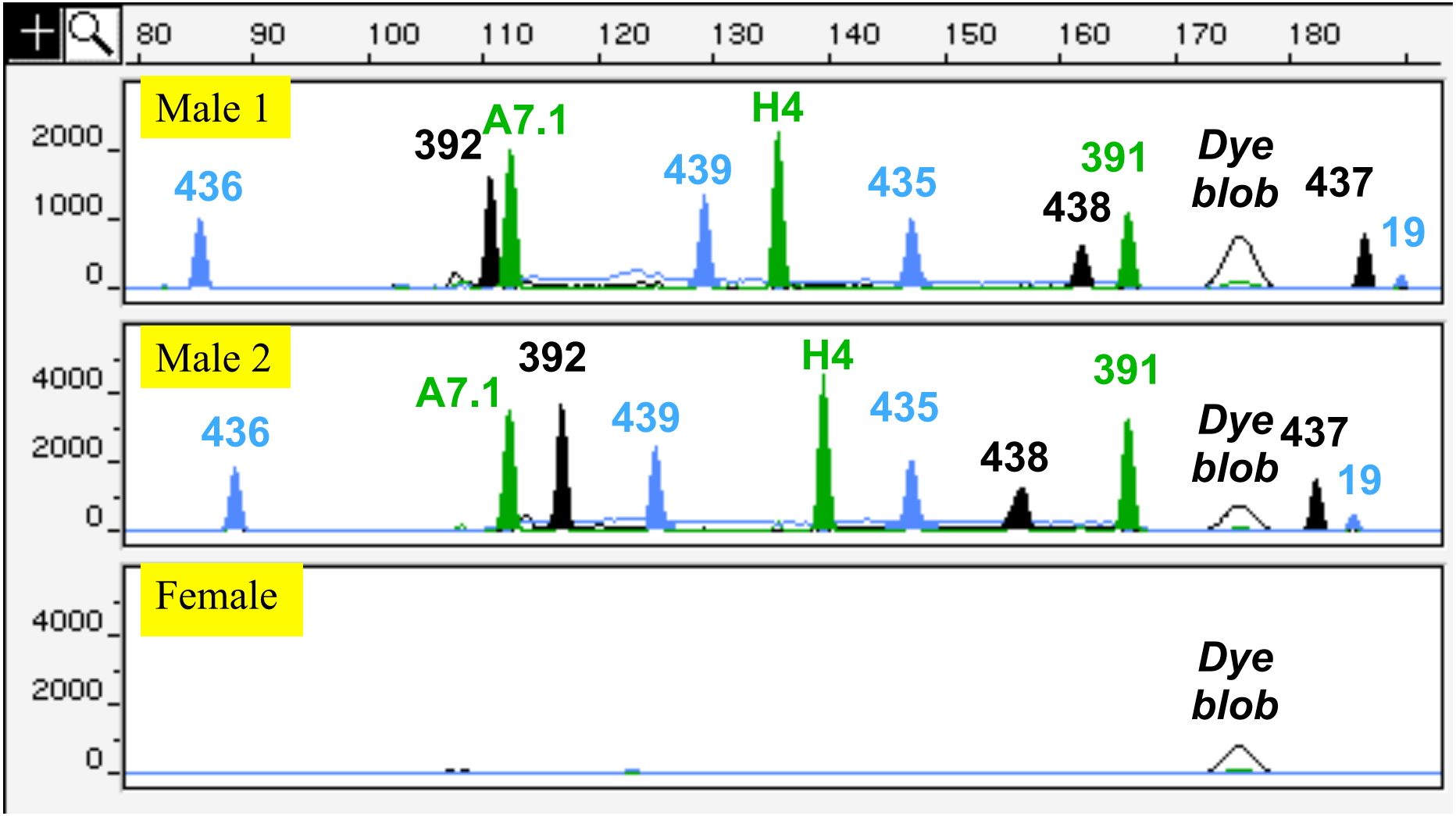


More markers = greater power of discrimination

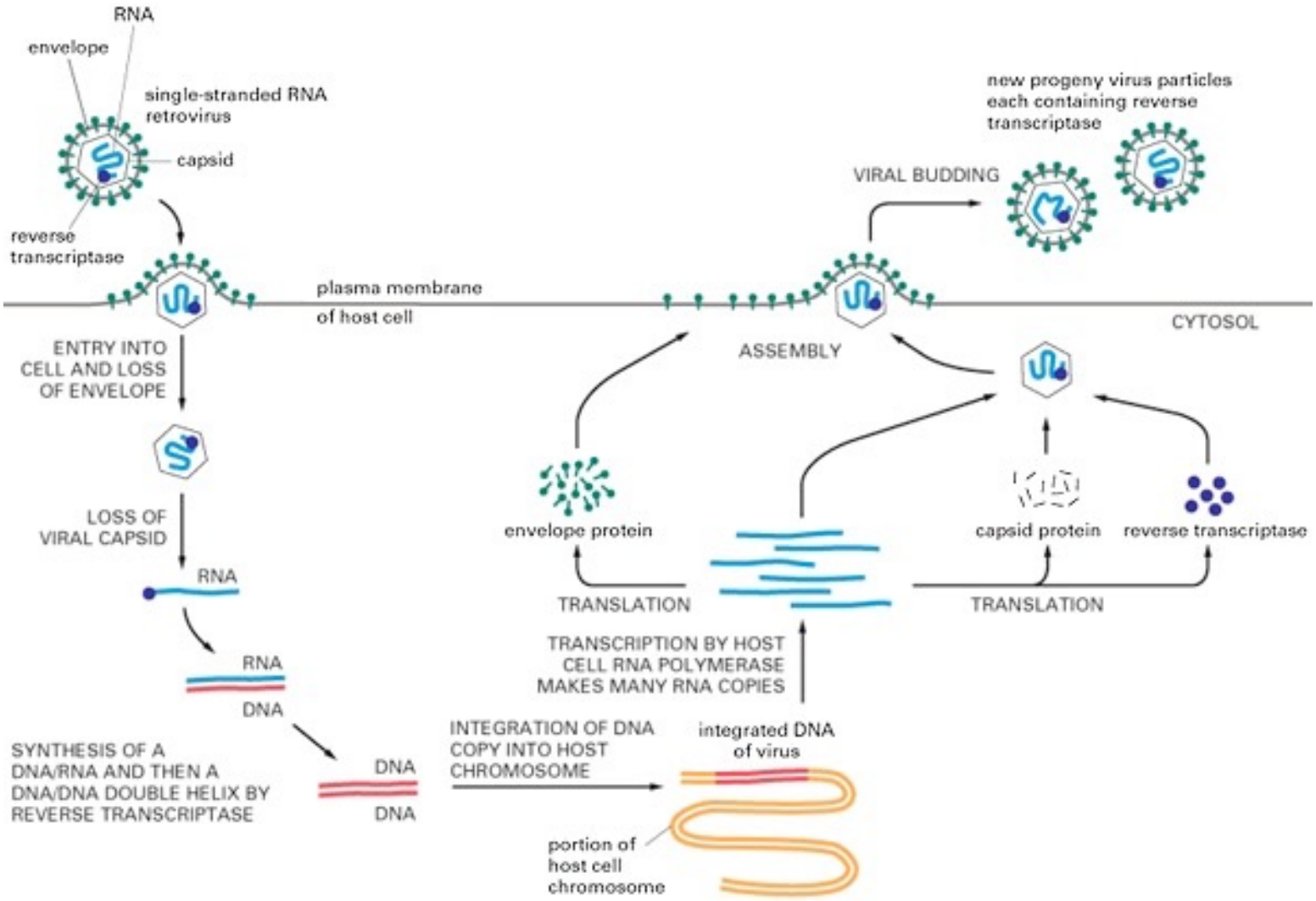
n = 850 mt-DNA D-Loop sequences (data kindly provided by the Institute of Legal Medicine Magdeburg, Germany)
n = 2196 extended European haplotypes logged in the database

YSTR DATABASE

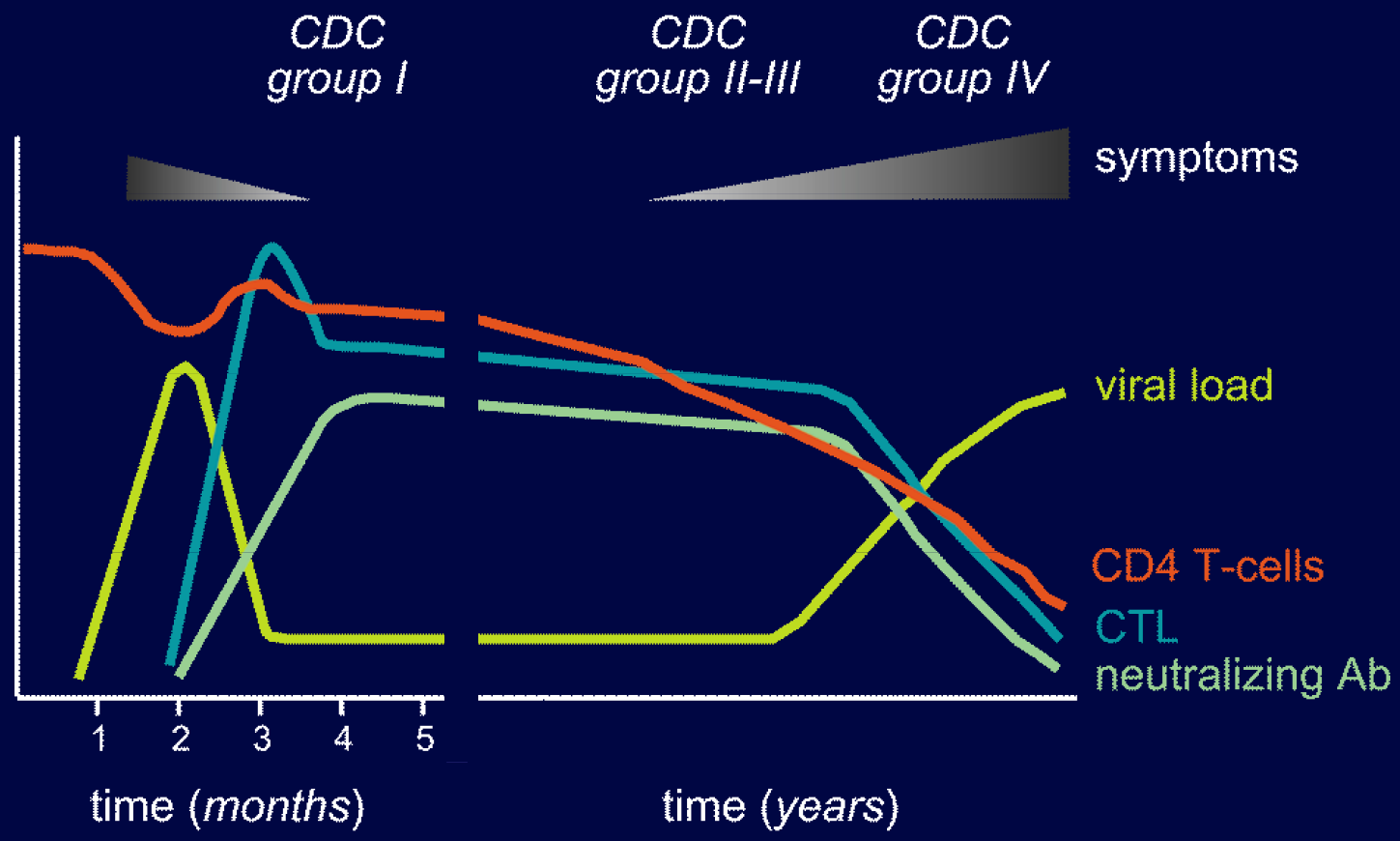
http://www.ystr.org/europe/


80
90
100
110
120
130
140
150
160
170
180
Male 1
2000
1000
0
436
392
A7.1
439
H4
435
438
391
Dye blob
437
19
Male 2
4000
2000
0
436
A7.1
392
439
H4
435
438
391
Dye blob
437
19
Female
4000
2000
0
Dye blob

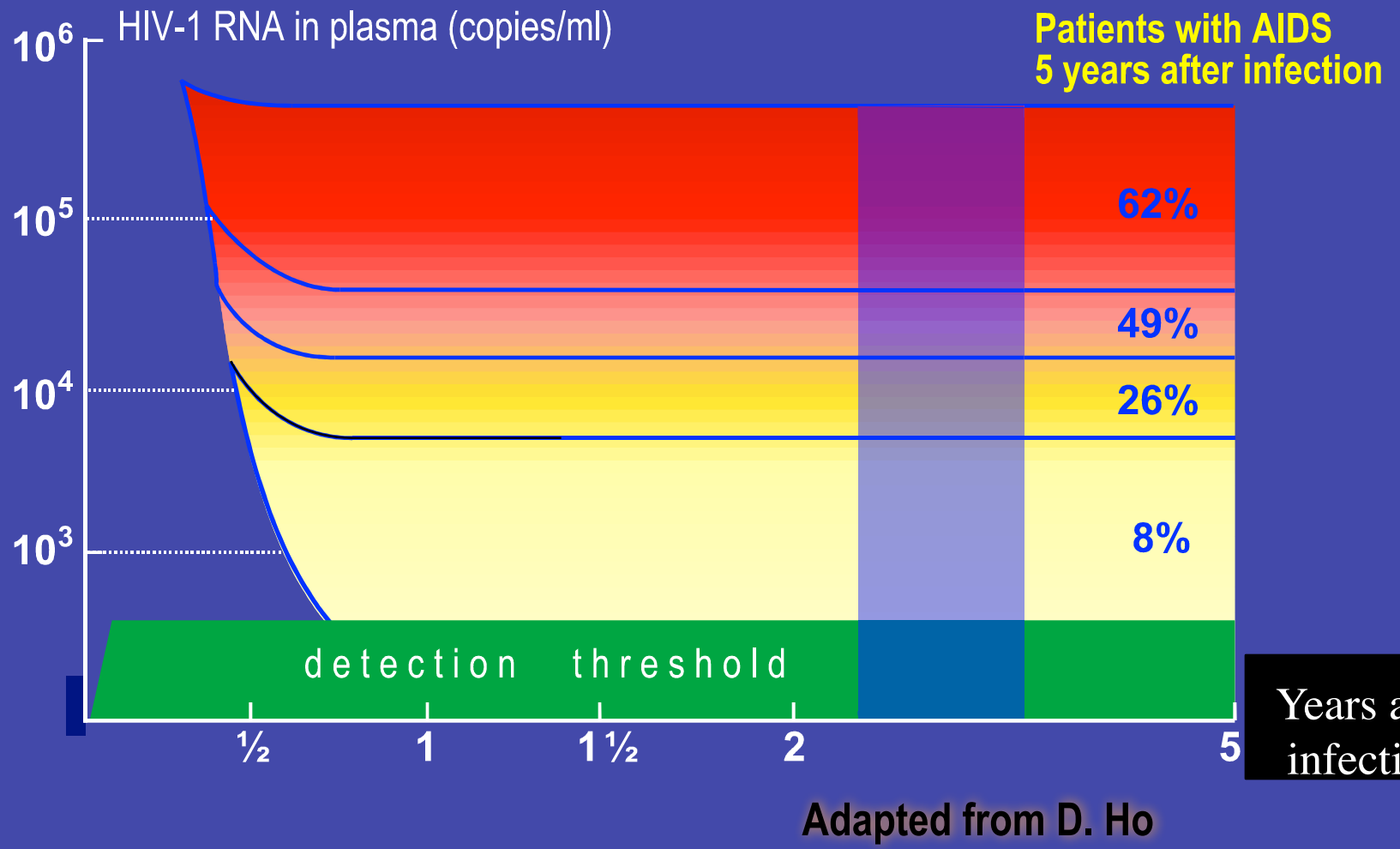
HIV life cycle



Natural history of HIV infection

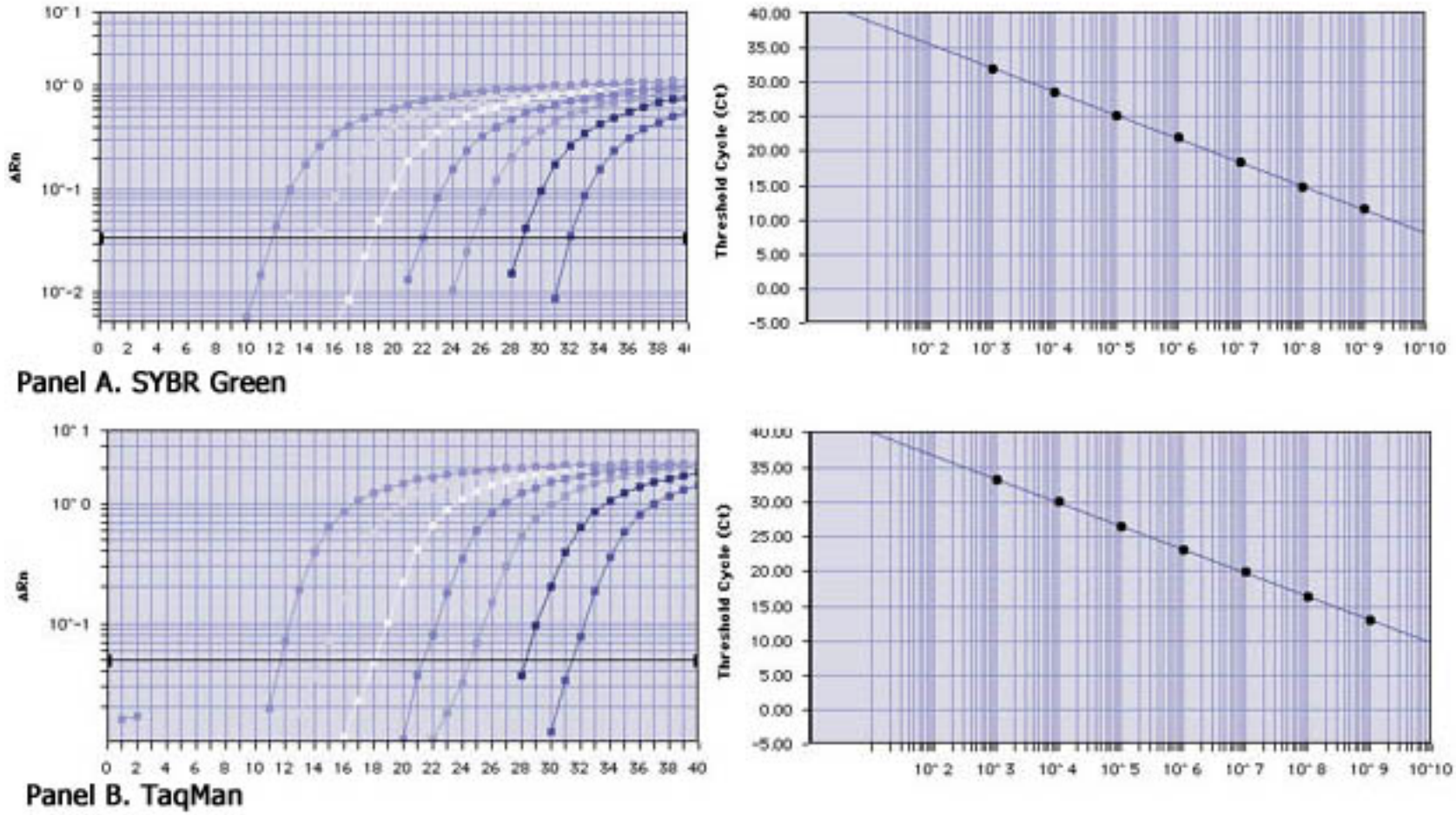


Correlation between HIV load in plasma and progression to AIDS



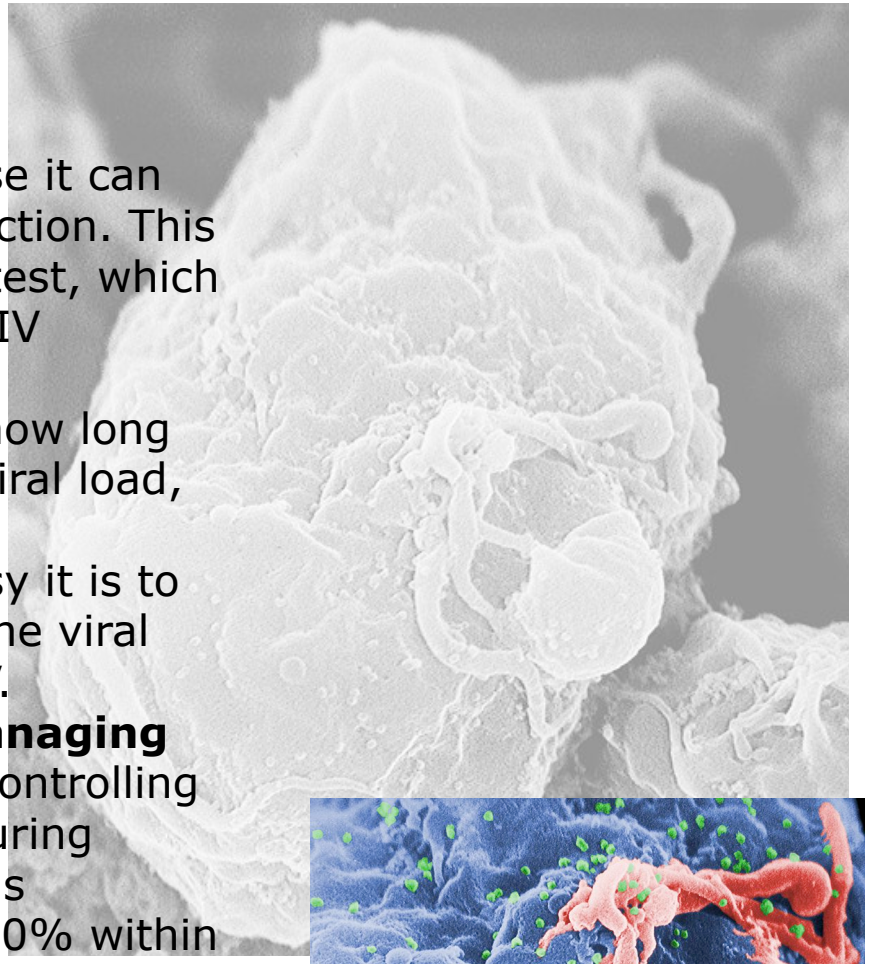
Adapted from D. Ho

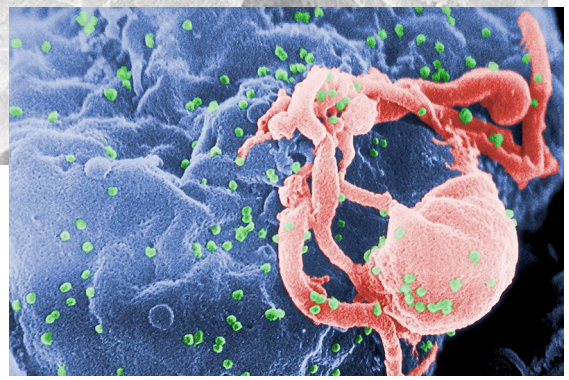
Precision and Accuracy of Real-Time RT-PCR. HIV amplification profiles and standard curves generated using A) SYBR Green, and B) TaqMan chemistries allow precise quantification of the viral load



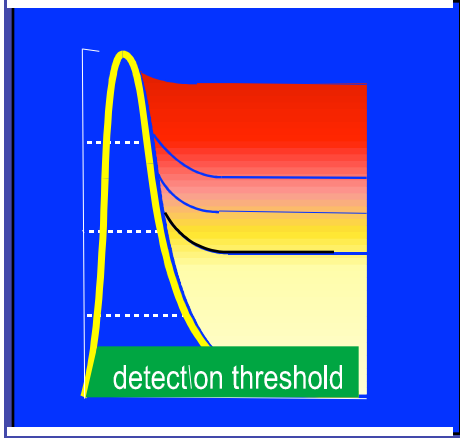
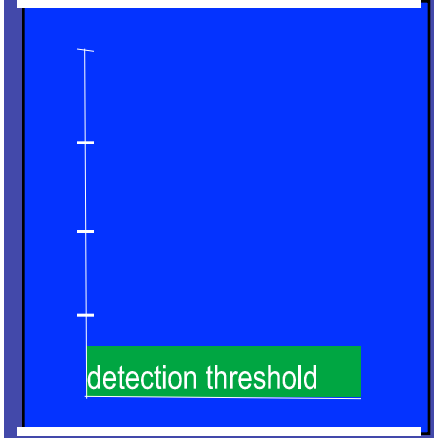
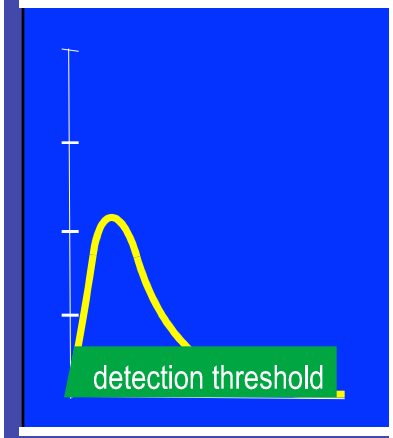
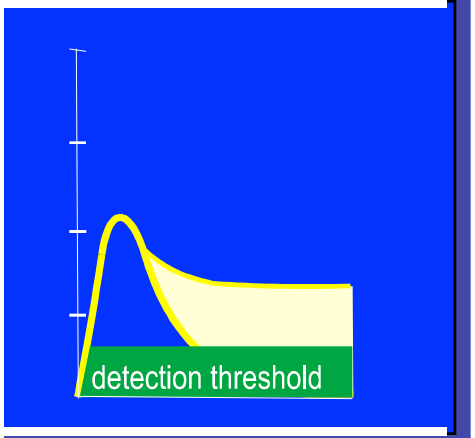
The HIV viral load is helpful in several areas:

- The test can be used for **diagnosis**, because it can detect a viral load a few days after HIV infection. This is better than the standard HIV (antibody) test, which can be "negative" for 2 to 6 months after HIV infection.
- For **prognosis**, viral load can help predict how long someone will stay healthy. The higher the viral load, the faster HIV disease progresses.
- For **prevention**, viral load predicts how easy it is to transmit HIV to someone else. The higher the viral load, the higher the risk of transmitting HIV.
- Finally, the viral load test is valuable for **managing therapy**, to see if antiretroviral drugs are controlling the virus. Current guidelines suggest measuring baseline (pre-treatment) viral load. A drug is "working" if it lowers viral load by at least 90% within 8 weeks. The viral load should continue to drop to less than 50 copies within 6 months. The viral load should be measured within 2 to 8 weeks after treatment is started or changed, and every 3 to 4 months after that.

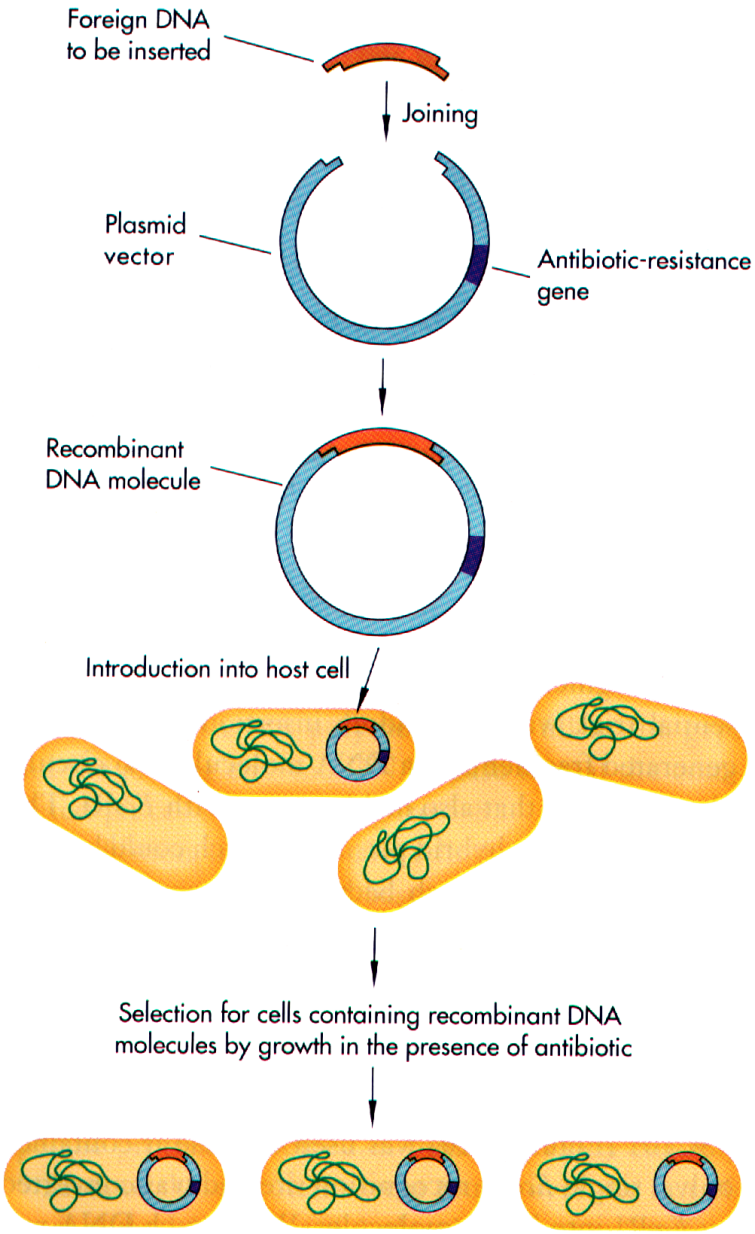




Potential end-points of HIV-vaccine efficacy trials

CONTROL (or ineffective vaccine)	PRIMARY END-POINT	SECONDARY END-POINTS	
no protection	protection against HIV (sterilizing immunity)	protection against disease (modification of the course of HIV infection in vaccine recip nts)	
			
"normal" infection with variable levels of viral load	*no infection*	*initial infection "controlled"*	*establishment of chronic infection with low viral l*

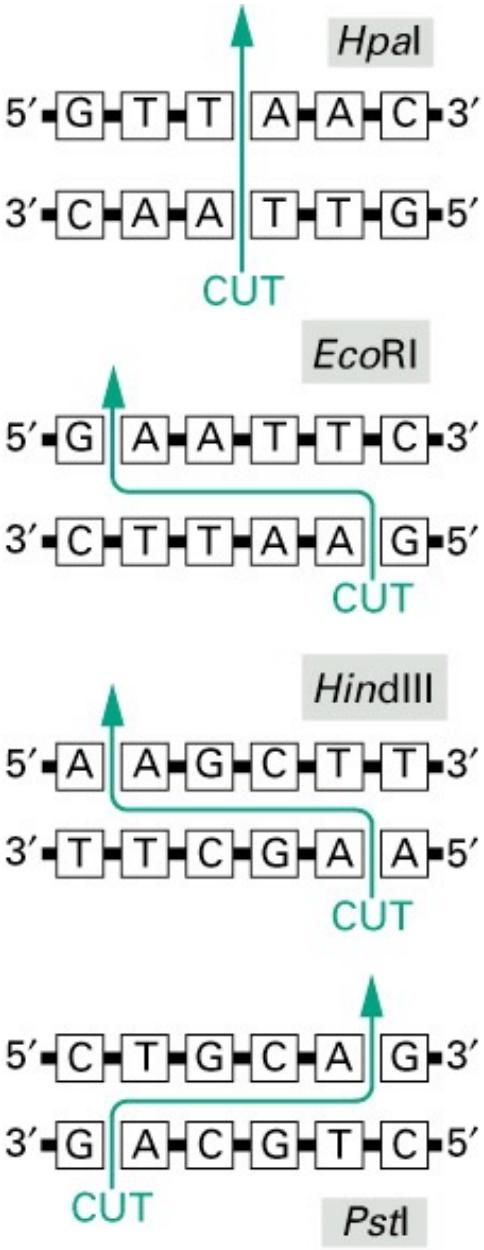
DNA cloning



The cloning of DNA in a plasmid.

The basic rule in genetic engineering is the universal use of the genetic code

Restriction enzymes



The Nobel Prize in Physiology or Medicine 1978
Werner Arber, Daniel Nathans, Hamilton O. Smith

The Nobel Prize in Physiology or Medicine 1978

Nobel Prize Award Ceremony

Werner Arber

Biographical
Nobel Lecture
Interview
Other Resources

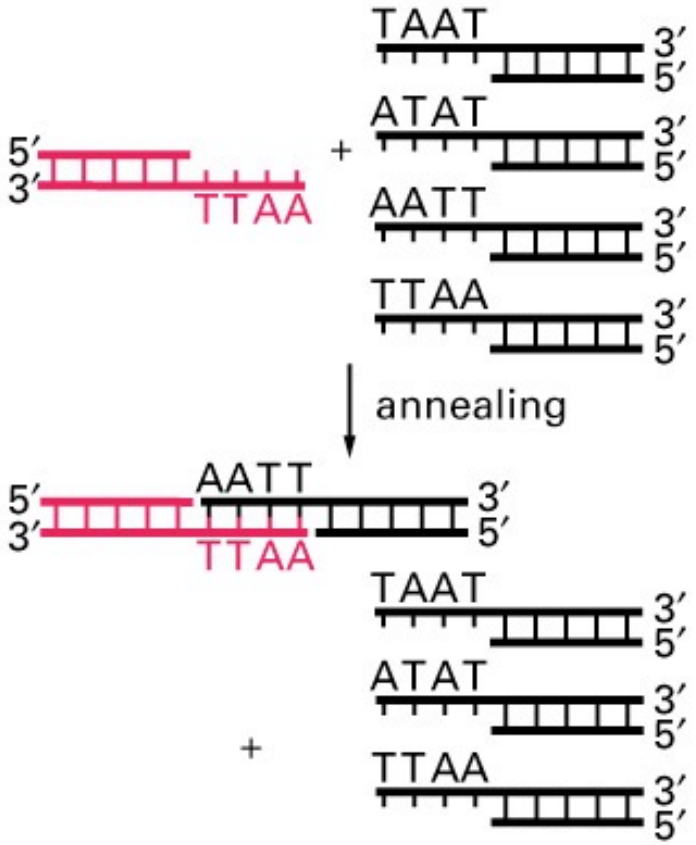
Daniel Nathans

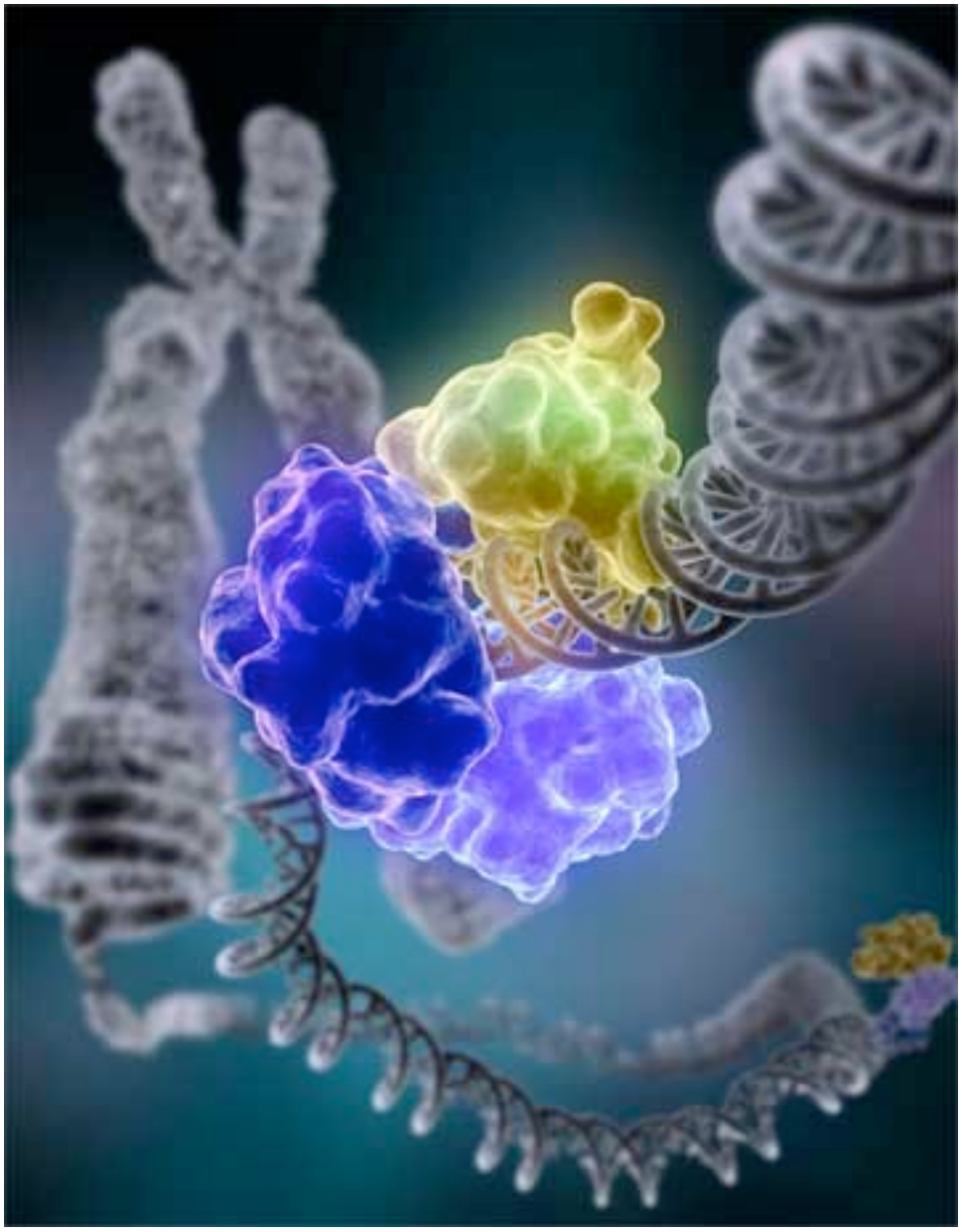
Biographical
Nobel Lecture
Banquet Speech

Hamilton O. Smith

Biographical
Nobel Lecture
Interview

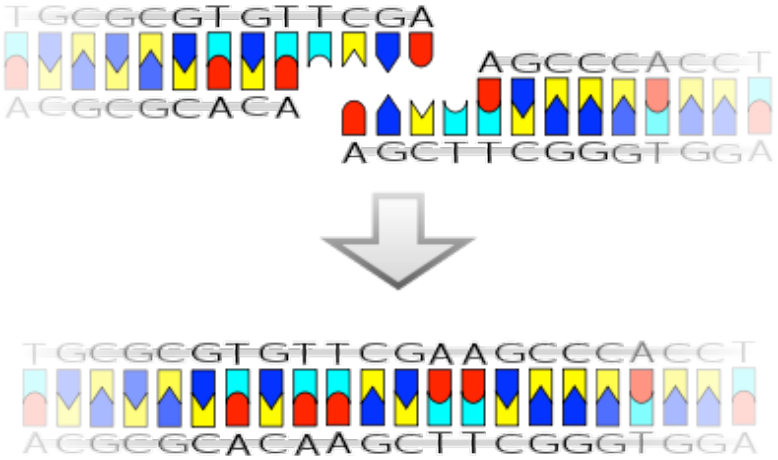
Recombinant DNA molecules





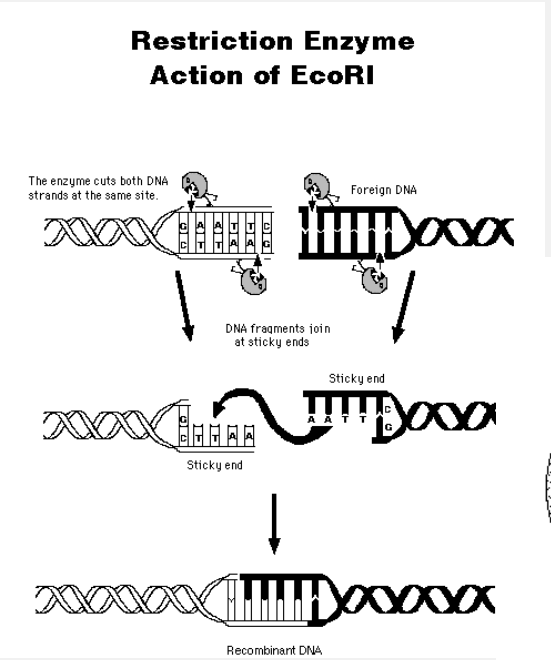
Ligase mechanism

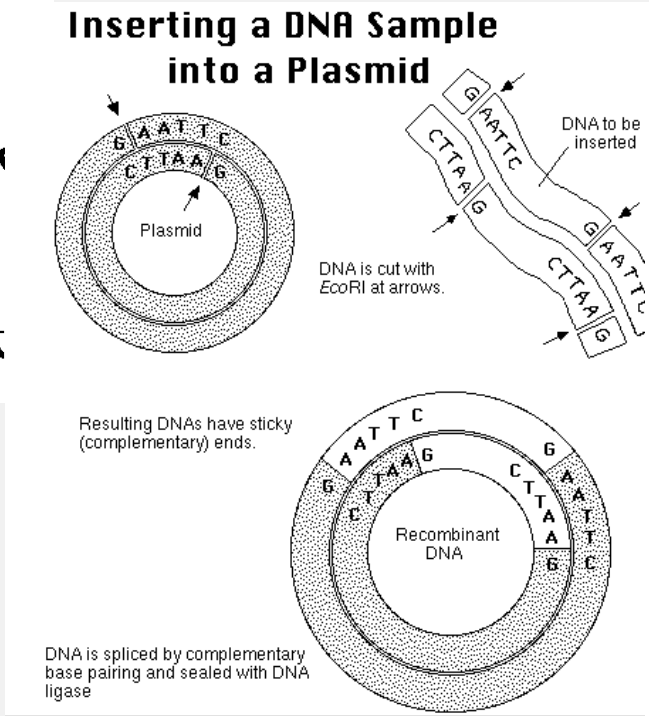
The mechanism of DNA ligase is to form two covalent phosphodiester bonds between 3' hydroxyl ends of one nucleotide with the 5' phosphate end of another. ATP is required for the ligase reaction.
A pictorial example of how a ligase works (with sticky ends):

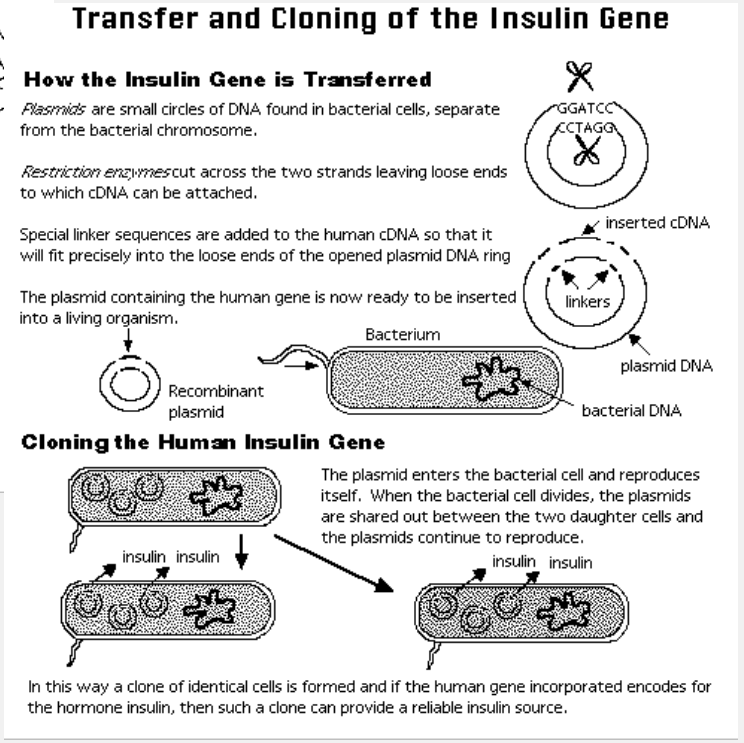


Ligase will also work with blunt ends, although higher enzyme concentrations and different reaction conditions are required.

Recombinant DNA technology







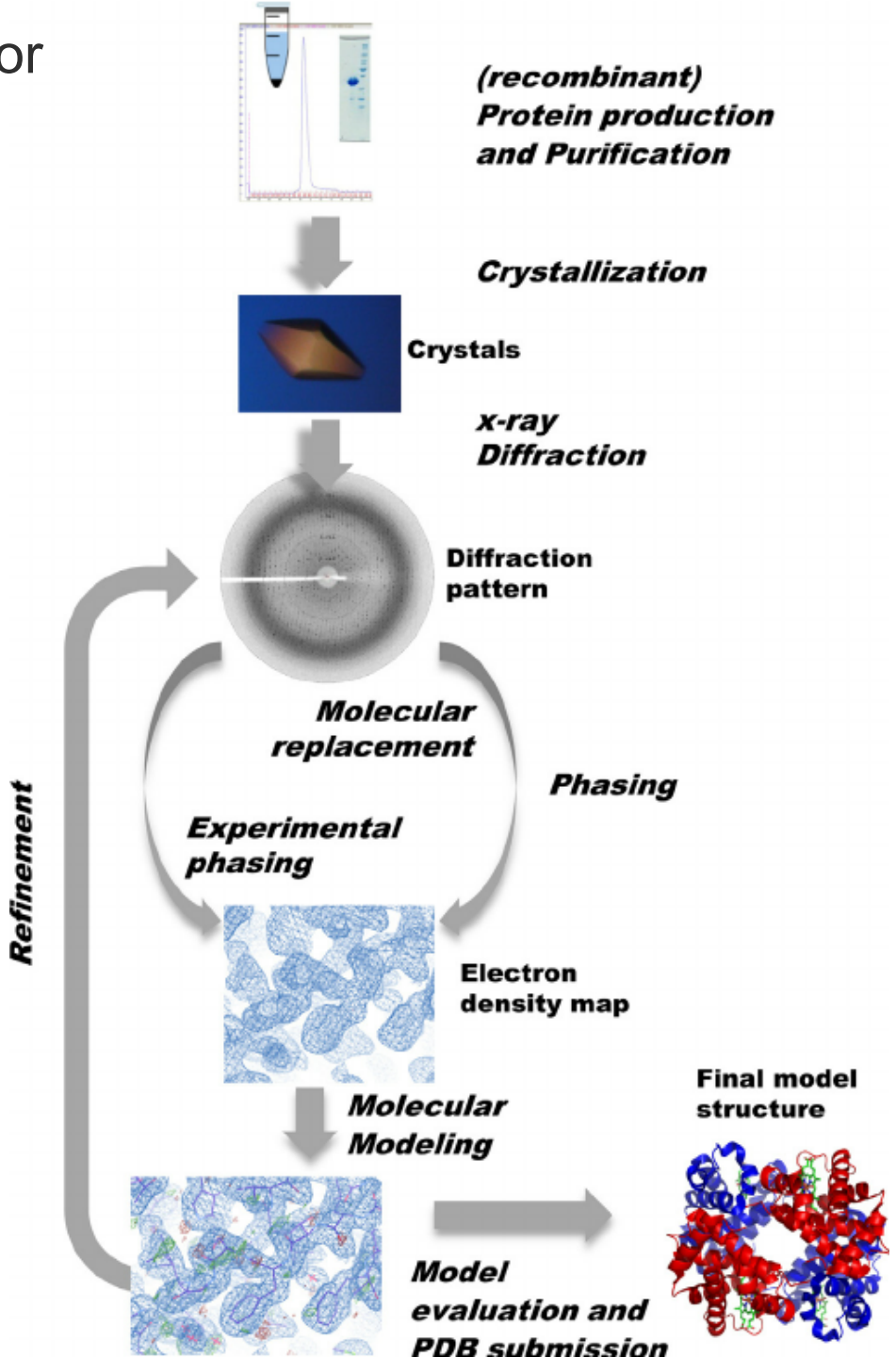
Biopharmaceutical Products

Product	*Product*
Insulin	1982
Human Growth Hormone (hGH)	1985
α–Interferon	1986
Hepatitis B Vaccine	1986
Tissue Plasminogen Activator (TPA)	1987
Erythropoietin-α	1989
γ-Interferon	1990
Granulocyte Colony Stimulating Factor (G-CSF)	1991
Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF)	1991
Interleukin 2	1992
Factor VIII	1992
β-Interferon	1993
DNase (Pulmozyme®)	1993
Glucocerebrosidase (Cerezyme®)	1994
ReoPro®	1994

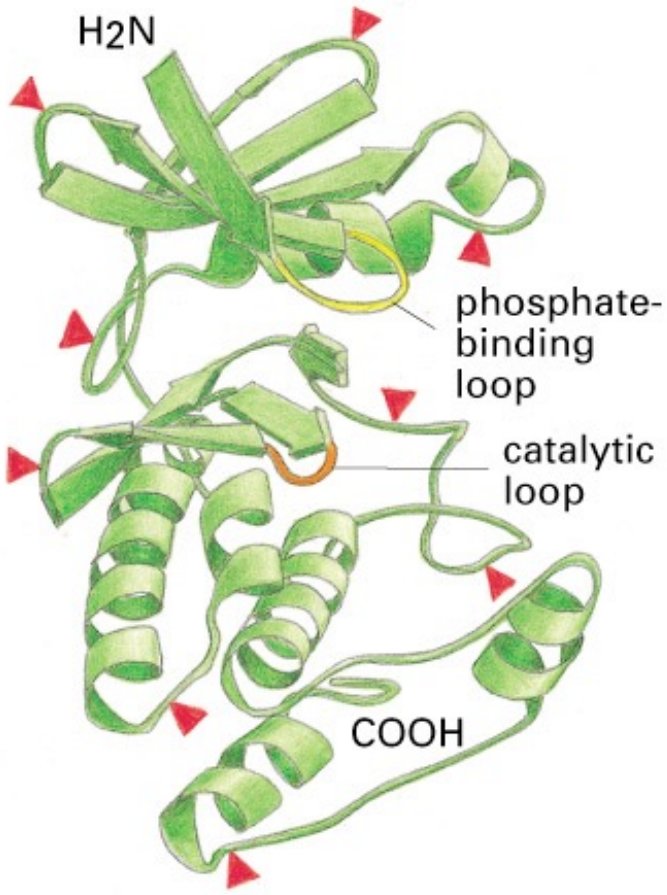
Source: Consulting Resources Corp.

The use of molecular engineering for protein structure determination

Recombinant techniques are routinely used for the preparation of protein samples for structural studies including X-ray crystallography. Among other benefits, these methods allow for a vast increase in the amount of obtained protein as compared to purification from source tissues, ease of purification when fusion proteins containing affinity tags are used, and the opportunity to modify the protein to enhance its crystallizability. Protein engineering may involve removal of flexible regions including termini and interior loops, as well as replacement of residues that affect solubility. Moreover, modification of the protein surface to induce crystal growth may include rational engineering of surface patches that can readily mediate crystal contacts. The latter approach can be used to obtain proteins of crystals recalcitrant to crystallization or to obtain well-diffracting crystals in lieu of wild-type crystals yielding data to limited resolution.



The 3-D structure of a protein kinase



Insertions of 5-100 aa in surface loops confer distinctive interactions with selected ligands

The ATP (which donates the P group) and the substrate are held in the active site, between the orange and yellow loops

DISCOVERIES LEADING TO FDA APPROVAL OF STI571/Gleevec FOR TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA

1960

1960 – Abnormal chromosome 22 (Philadelphia Chromosome) observed in CML patients

1970

1973 – Chromosome 22 and 9 translocation observed by new staining techniques

1980

1982 – *abl* Proto-oncogene identified in chromosome 22 translocation

1984-1987 – BCR-ABL protein identified as possible cause of CML

1990

1990 – *bcr-abl* Gene identified as cause of leukemia in mice

1993 – First STI571/Gleevec laboratory studies begin

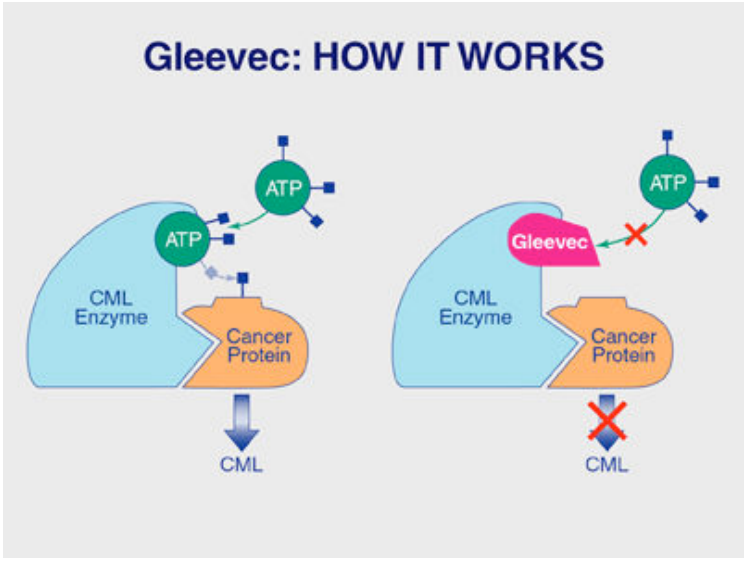
1998 – First human tests begin

1999 – First human results reported

2000

2001 – April: Larger study confirms earlier findings

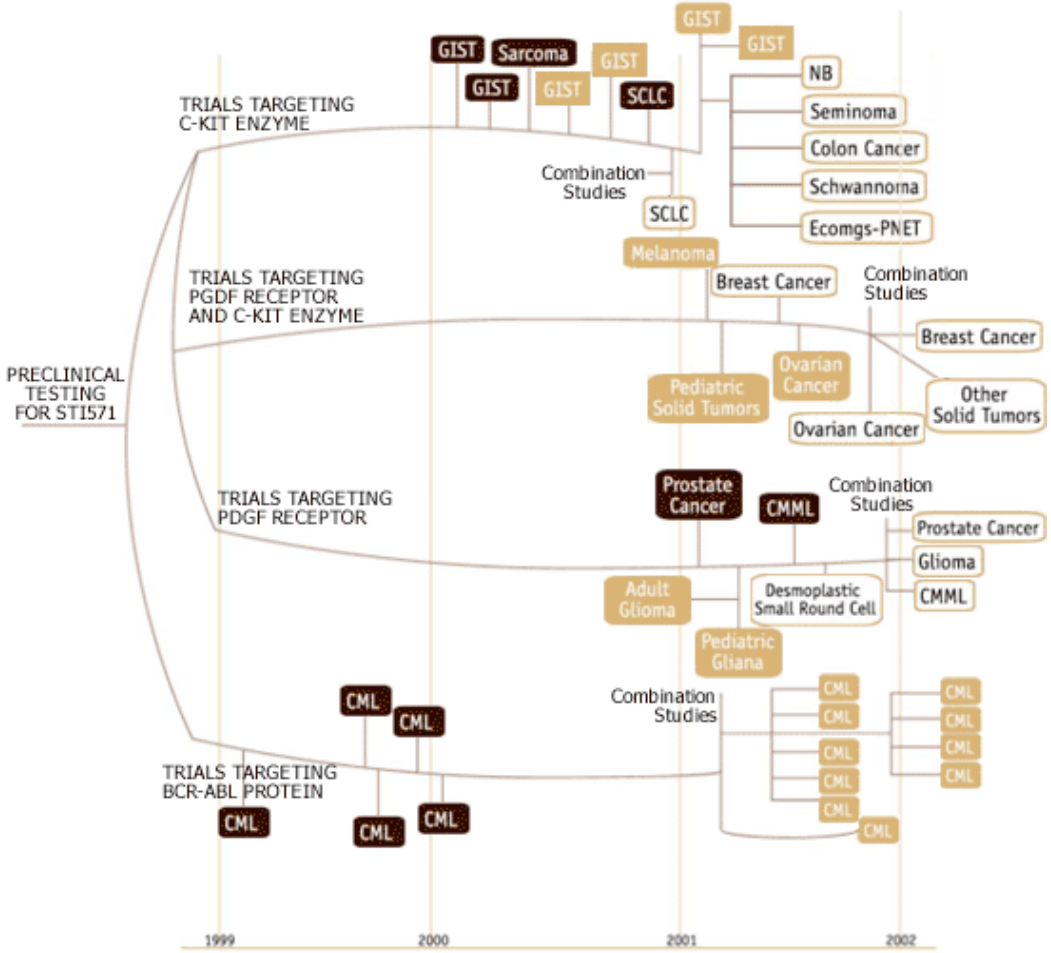
2001 – May: FDA approves STI571/Gleevec for treatment for CML




HHMI

Clinical Trials for STI571 Have Mushroomed Since Early 1999

After success with a small Phase I clinical trial to test the safety of STI571 (Gleevec™) for treating chronic myelogenous leukemia, clinical investigators began testing the drug in a variety of cancers that share common molecular abnormalities. A rapid and broad expansion of clinical trials followed.



NCI Sponsored

Company Sponsored

NCI, Company, or Both

Boxes with rounded corners indicate Phase I or II preliminary clinical trials to assess factors such as toxicity, dosage, and activity of a new drug.

Boxes with square corners indicate Phase III or larger, more in-depth trials that, for example, look at long-term effects to compare the drug to standard therapies.

GIST
Gastrointestinal stromal tumors

CML
Chronic Myelogenous leukemia

CMML
Chronic myelomonocitic leukemia

SCLC
Small cell lung cancer

NB
Neuroblastoma

Tyrosine kinase inhibitors

Tyrosine kinase inhibitors are also called TKIs. They block chemical messengers (enzymes) called tyrosine kinases. Tyrosine kinases help to send growth signals in cells. So blocking them stops the cell growing and dividing. Cancer growth blockers can block one type of tyrosine kinase or more than one type. TKIs that block more than one type of tyrosine kinase are called multi-TKIs.

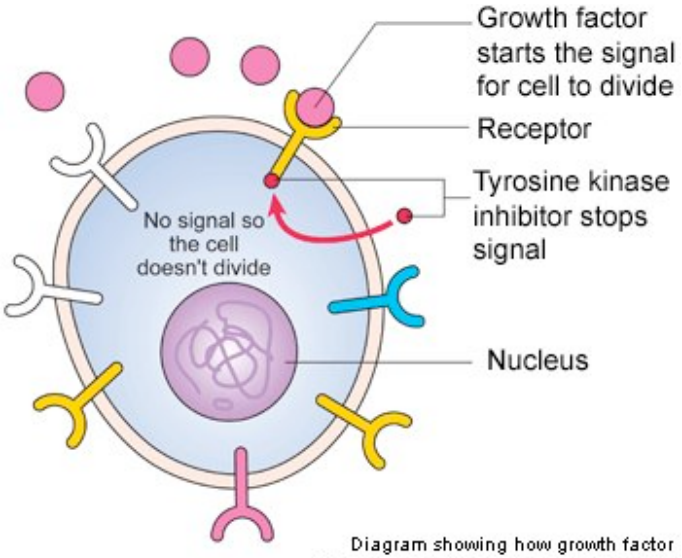


Diagram showing how growth factor inhibitors stop the signal inside the cell
Copyright © CancerHelp UK

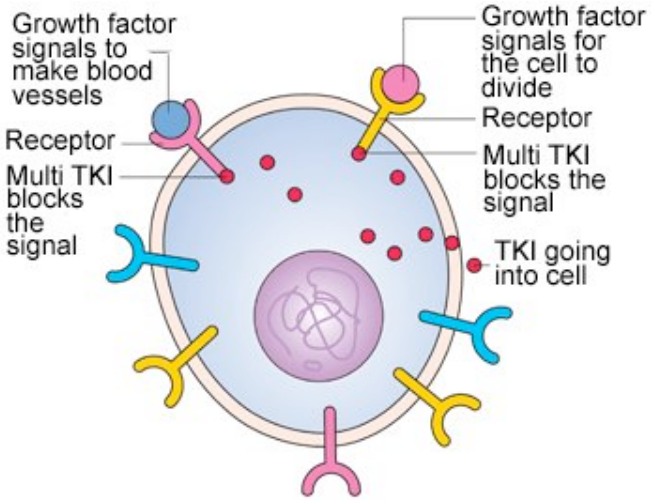
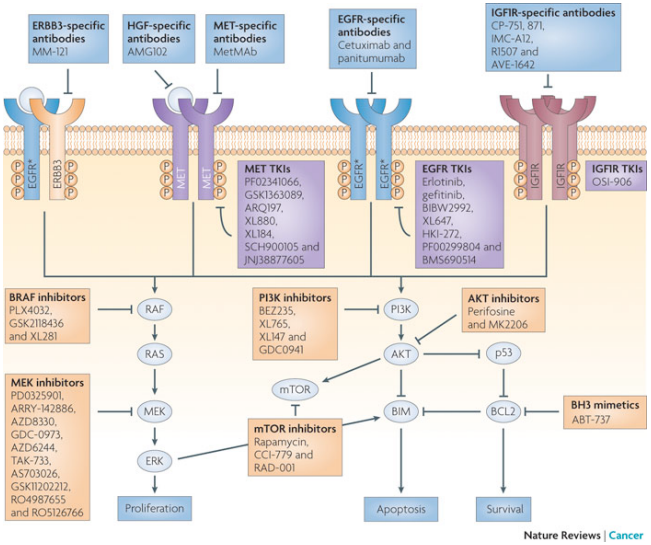


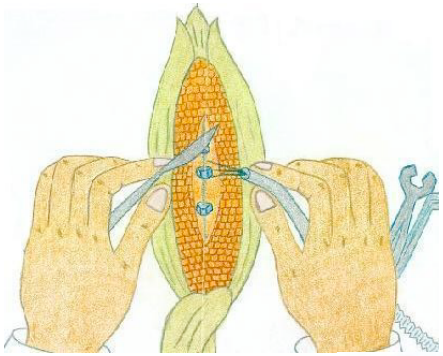
Diagram showing an example of how growth inhibitors can block more than one action in a cell (multi TKI)
Copyright © CancerHelp UK



TKIs Approved for Clinical Use

- Herceptin (trastuzumab) - metastatic breast cancer
- Glivec (imatinib) - chronic myeloid leukaemia and GIST
- Irressa (gefitinib) - NSCLC
- Erbitux (cetuximab) - metastatic colorectal cancer
- Tarceva (erlotinib) - NSCLC

Genetically Modified Organisms



Tolleranza zero sugli **immigrati** ma non sugli **O**rganismi **G**eneticamente **M**odificati

letto su *'L'Unità on line'*

GMOs

A genetically modified organism, or GMO, is an organism that has had its DNA altered or modified in some way through genetic engineering.

By far the biggest use of GMO technology has been in large-scale agricultural crops: At least 90 percent of the soy, cotton, canola, corn and sugar beets sold in the United States have been genetically engineered. The adoption of herbicide-resistant corn, which had been slower in previous years, has accelerated, reaching 89 percent of U.S. corn acreage in 2014 and in 2015, according to the U.S. Department of Agriculture.

Vocal anti-GMO activists — who refer to GMO crops as "Frankenfoods" — argue that GMOs can cause environmental damage and health problems for consumers.

The perception that GMOs are **not natural** has resulted in more than 50 legal cases involving food companies being sued for placing the word natural on its labels for food products that contain GM ingredients.

Many crops never existed in nature

Fragraria chiloensis
Chile

X

Fragraria virginiana
Eastern North America

Fragraria ananassa
Paris Botanical Garden, 1766

Seedless fruits are not natural

Citrus farmers grow seedless fruits, such as navel oranges and clementines, all over the world

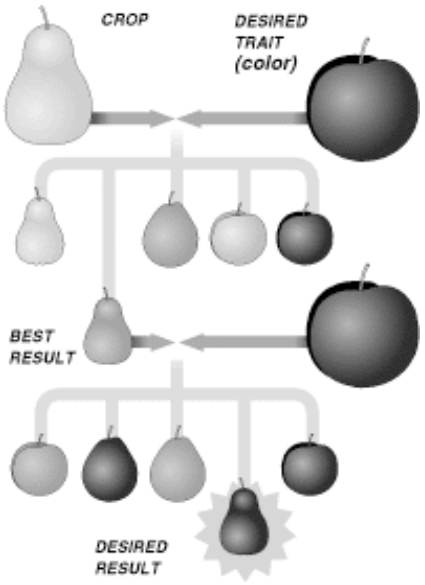
An Assyrian relief carving from 870 B.C. showing artificial pollination of date palms.

http://www.colostate.edu/programs/lifesciences/TransgenicCrops/history.html

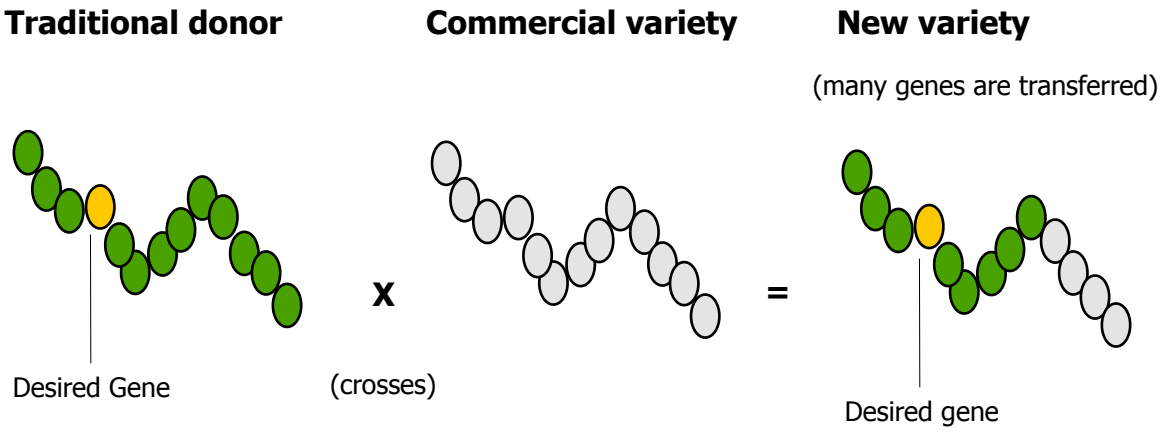
More than one way to alter a plant

Traditional plant breeding

For centuries, when farmers wanted to introduce a new trait to their favourite crops (making them more durable, productive or marketable), they would breed the crop with a plant possessing the desired characteristic.
In trying to get a pear with a coloration of an apple, they might crossbreed their preferred pear with a chosen variety of apple. This would produce a range of hybrids with combinations of the characteristics of both fruits. Of those, the fruit closest to the desired result would be chosen and bred again with the apple. This process would be repeated over many generations until the desired trait was achieved.
Only traits from species that are relatively close to one another can be combined in this way. This process can take months to years to produce the desired results.



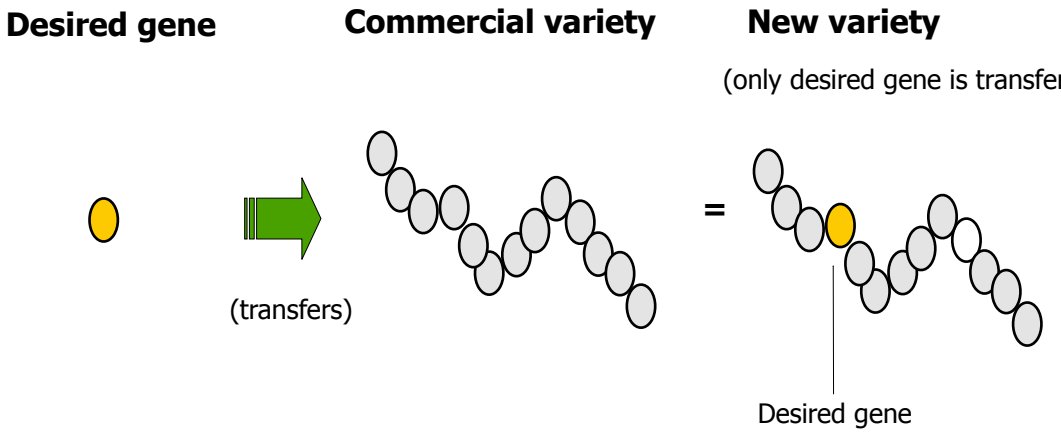
DNA is a strand of genes, much like a strand of pearls. Traditional plant breeding combines many genes at once.



More than one way to alter a plant

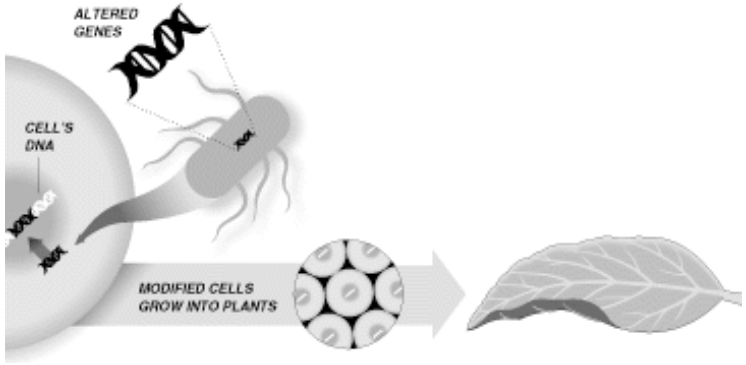
Plant biotechnology

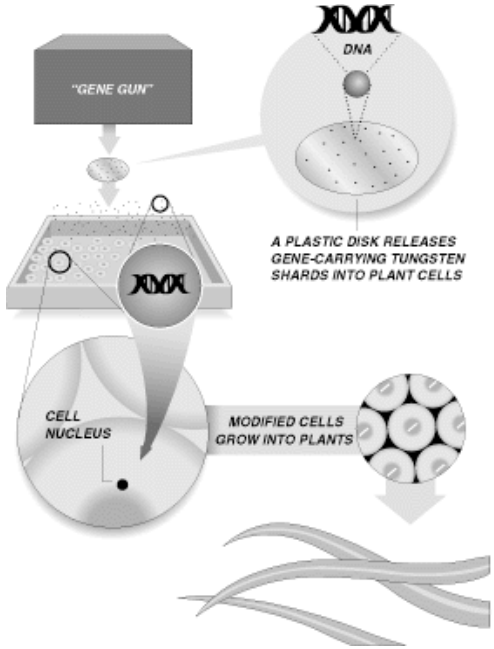
Using plant biotechnology, a single gene may be added to the strand.



There are two common methods for introducing the genetic material responsible for a desired trait in to a given crop:

1. The first uses Agrobacterium, a bacterium that naturally alters a plant's DNA. Placing the desired genes into the bacterium and then infecting the plant, it inserts the new genetic codes into the plant's DNA. The cells are then grown to maturity, producing future generations with the desired characteristics





2. The second method uses a "gene gun" to propel genetic material coating thousands of microscopic shards of tungsten into a group of plant cells. The tungsten penetrates the cells and carries the DNA to the nucleus.
Using this strategy, traits can be bred more accurately, but some complex traits remain difficult if not impossible.

Why do we need GMOs?

Protect crops from biotic and abiotic stresses

Reduce environmental footprint of agriculture

Improved nutrition

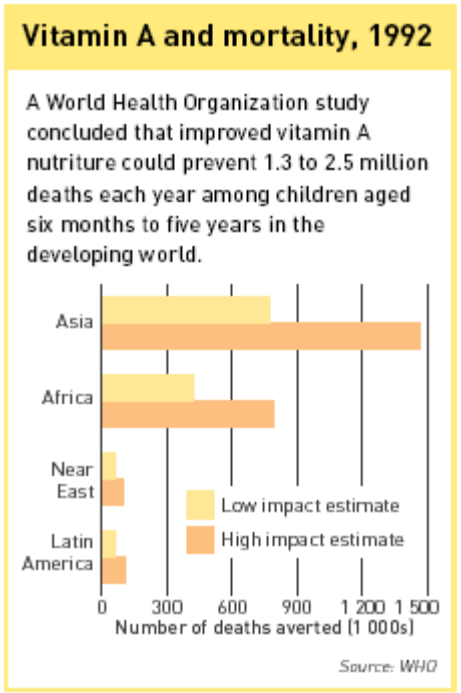
Improved quality and consumer appeal

Bioremediation

Plants and animals as Factories

Nutritionally Enhanced: Golden Rice

Vitamin A deficiency is a leading cause of blindness often leading to mortality throughout the developing world. Every year 2 million children, mostly in developing countries, either die or suffer from developmental defects because they are deficient in vitamin A. Without vitamin A proper development does not take place and children frequently die by the age of 4 or 5. If we compare this with acquired immune deficiency syndrome (AIDS), tuberculosis or malaria, there are many more children dying from vitamin A deficiency.



The solution: golden rice, engineered to produce beta-carotene, the precursor of vitamin A

Against the grain

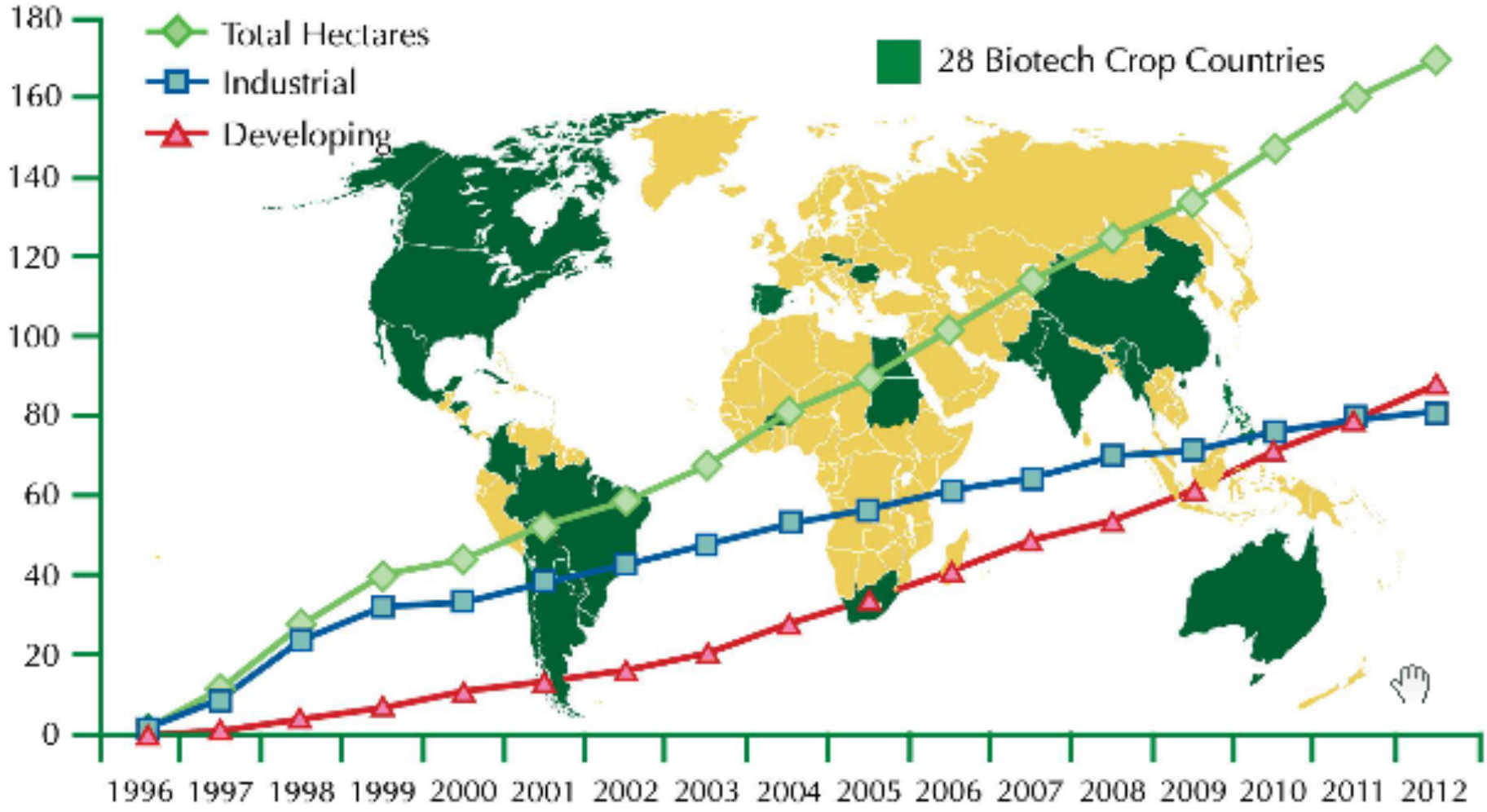
Golden rice could help to end a nutritional crisis — but only if researchers can overcome some daunting technical and political hurdles.

Unfortunately, the golden rice strain selected for field testing does not grow as well as local rice varieties, limiting its appeal to struggling farmers. "The final product has to be so good that it will be readily adopted by farmers in terms of agronomic traits — yield, disease resistance, quality and ability to withstand adverse conditions — as well as β-carotene production," says Antonio Alfonso of PhilRice, who led the trials.

The scientific problems can be solved, but public fears over GM organisms (GMOs) may be a bigger obstacle. Activists in Europe and North America have shaped the debate by raising doubts and concerns over the environmental impact and health risks of 'unnatural' GMOs, even though scientists have pointed to numerous studies that should assuage these worries.

Pope Blesses Golden Rice

GLOBAL AREA OF BIOTECH CROPS
Million Hectares (1996-2012)



A record 17.3 million farmers, in 28 countries, planted 170.3 million hectares (420 million acres) in 2012, a sustained increase of 6% or 10.3 million hectares (25 million acres) over 2011.

La patata in Australia

PLRV (Potato Leaf Roll Virus) e' il principale problema per le coltivazioni di patate in Australia

Inserendo un gene di PLRV la patata diventa resistente all'infezione

Trasporto e lavorazione innescano un processo di ossidazione che conferisce alle patate uno sgradevole colore brunastro

Tale processo puo' essere prevenuto inserendo una copia antisenso del gene responsabile, la PPO (polifenol-ossidasi)

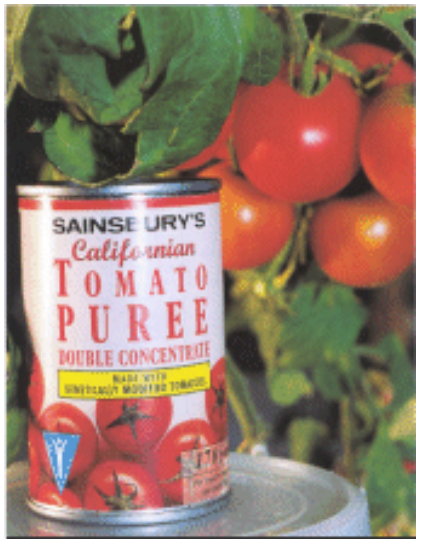
Le patate geneticamente modificate, resistenti all'infezione da PRLV e all'ossidazione, sono disponibili sul mercato australiano dall'anno 2000

In Europa ad un acceso dibattito intorno ai rischi degli OGM non corrisponde una adeguata conoscenza del problema dal punto di visto scientifico

- 35% della persone intervistate ritiene che i pomodori non contengano geni, mentre li contengono soltanto quelli geneticamente modificati

- il 24% ritiene che “Se una persona mangia OGM l geni si trasferiscano a lui”

Eurobarometer Survey, 2000



Are GMOs dangerous or safe?

Governments and government regulators around the world have approved dozens of GMO crop varieties on a CASE-BY-CASE basis

National Academies of Science, Royal Societies, Scientific societies, and Medical Societies around the world say they are safe

Careful pre-market scrutiny led to global post-market successes

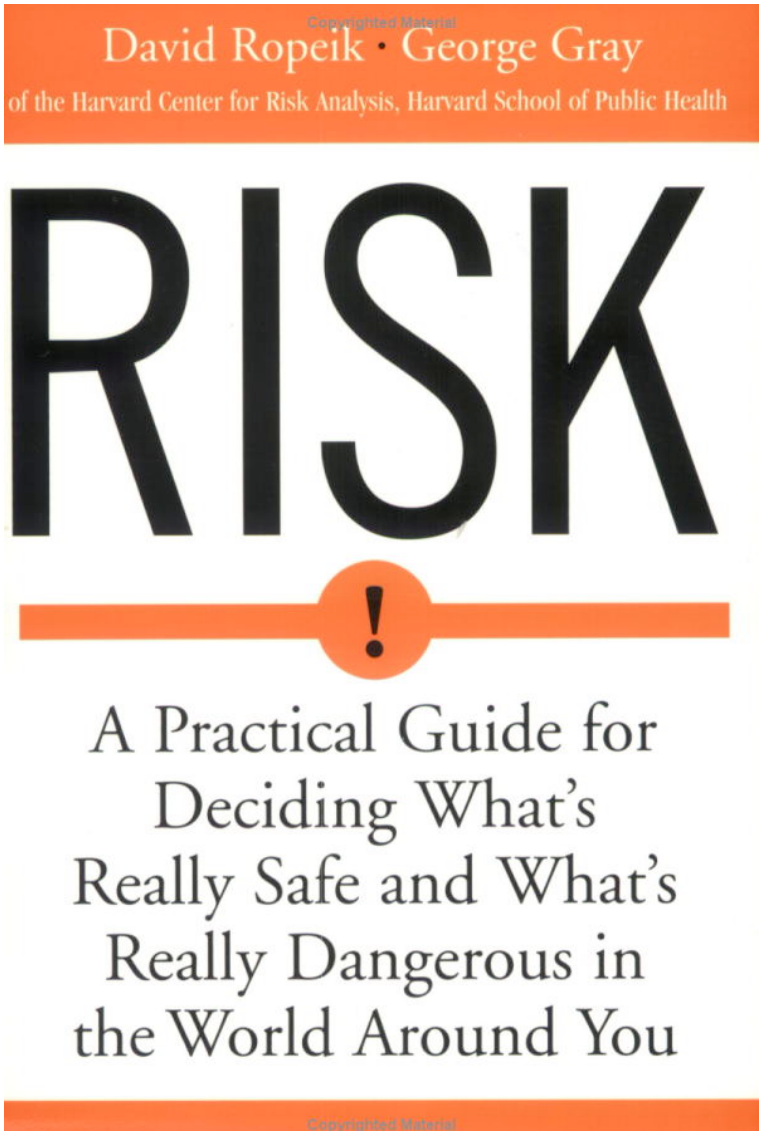
400,000,000 ha in 10 years, 17 countries, 8.5 million farmers

Recent publications from the US National Academy of Sciences and from EU scientists reported that GM foods "are as safe as or safer than" conventional foods

There is no real scientific controversy about GM food safety

The anti-GMO movement has managed to misrepresent the science to the media to create unscientific fear the world over

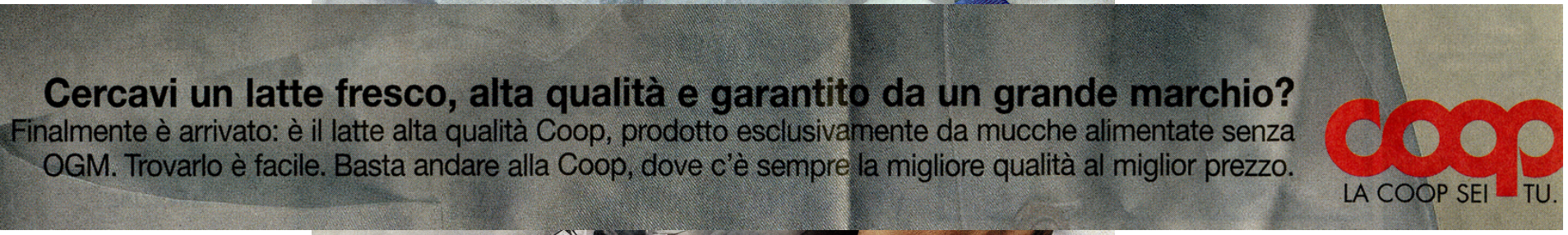
Years of research, thousands of scientific publications, careful review by government regulators make these the safest foods on the market

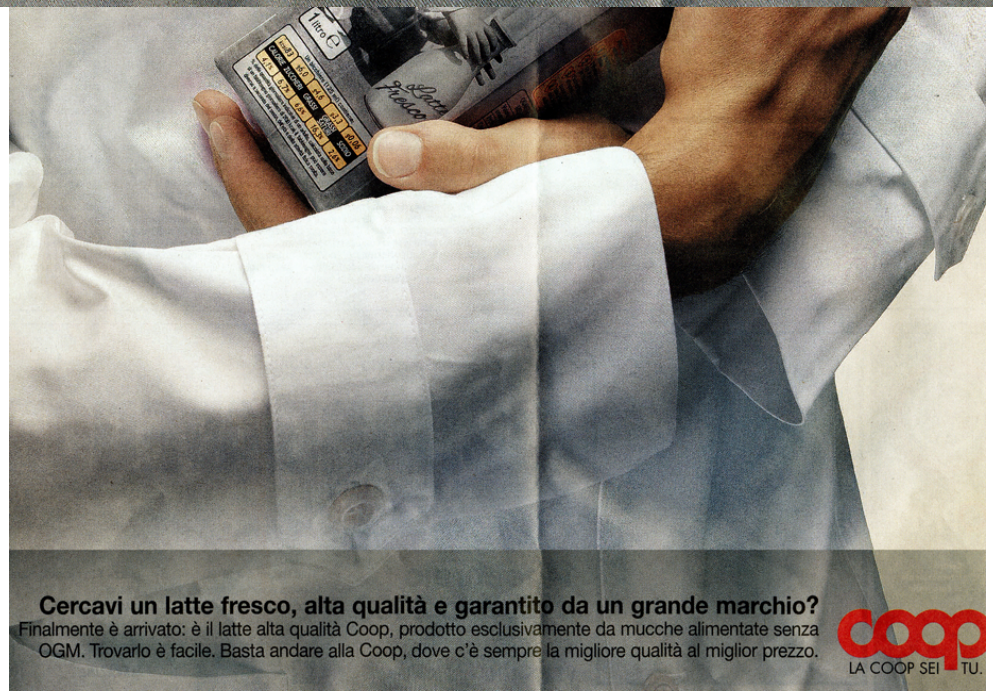


http://www.hcra.harvard.edu/risk.html
http://www.hcra.harvard.edu/


Nasce il latte fresco alta qualità Coop.
A soli 1,15 € al lt.
coop


Biological food
Download from
Dreamstime.com


Cercavi un latte fresco, alta qualità e garantito da un grande marchio?
Finalmente è arrivato: è il latte alta qualità Coop, prodotto esclusivamente da mucche alimentate senza OGM. Trovarlo è facile. Basta andare alla Coop, dove c'è sempre la migliore qualità al miglior prezzo.
coop
LA COOP SEI TU.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LA COOP SEI TU.


ENVIRONMENT FRIENDLY
100%
ORGANIC
NO HARM TO NATURE

Biological food can be a relevant source of poisoning

Corriere della Sera Sabato 22 Novembre 2008

La festa Indagine dell'Asl, 3 sono bambini

Pisa, nove intossicati alla rassegna del cibo bio

PISA — Almeno nove intossicati, tre dei quali (bambini dai 3 ai 10 anni) finiti all'ospedale con vomito e disturbi intestinali. La «due giorni» dedicata al cibo naturale, senza conservanti e fitofarmaci, si è conclusa con un'indagine dell'Asl e gravi ombre sugli organizzatori.

È accaduto a Pisa durante la rassegna «Dalla stalla alla tavola» patrocinata da Comune, Provincia, Camera di commercio, Federconsumatori e persino della Società della salute, organismo regionale nato all'interno delle Asl per promuovere il vivere sano e genuino. Ed è proprio l'Asl che sta indagando per accertare la natura dell'intossicazione. «Quasi certamente di origine alimentare — spiega la dottoressa Eleonora Virgone — anche se avremo dati certi solo la prossima settimana».

La kermesse alimentare non avrebbe avuto neppure il permesso dell'Asl, obbligatorio per legge. «Nessuno ci ha segnalato l'iniziativa e dunque non sono stati fatti i controlli preventivi», ha confermato Virgone.

Tra le persone intossicate Antonio Mosca, un dirigente d'azienda, finito all'ospedale con due figli piccoli: «Avevamo mangiato formaggio biologico e salame di agnello — spiega — siamo arrivati al pronto soccorso con un principio di disidratazione. Poi le cose sono migliorate, ma ho perso quattro chili e sino a ieri avevo giramenti di testa».

La festa aveva un'appendice dedicata ai bambini. Con tanto di novella dedicata alla fata Sementina, metafora dell'iniziativa, custode di un «orto magico tempestato di verdure buone e belle» minacciato da «un orco chiamato Veleno...». Che sembra aver avuto la meglio, rovesciando, nella realtà, il lieto fine della favola. Sempre che le analisi dell'Asl confermino la tesi dell'intossicazione alimentare. «Ipotesi molto probabile — dicono gli esperti — tutte le persone colpite hanno assaggiato cibi presenti alla rassegna».

Marco Gasperetti

I WON'T EAT ANYTHING THAT'S GENETICALLY MODIFIED...
IT COULD BE UNHEALTHY..
CHIPS
NICHOLSON
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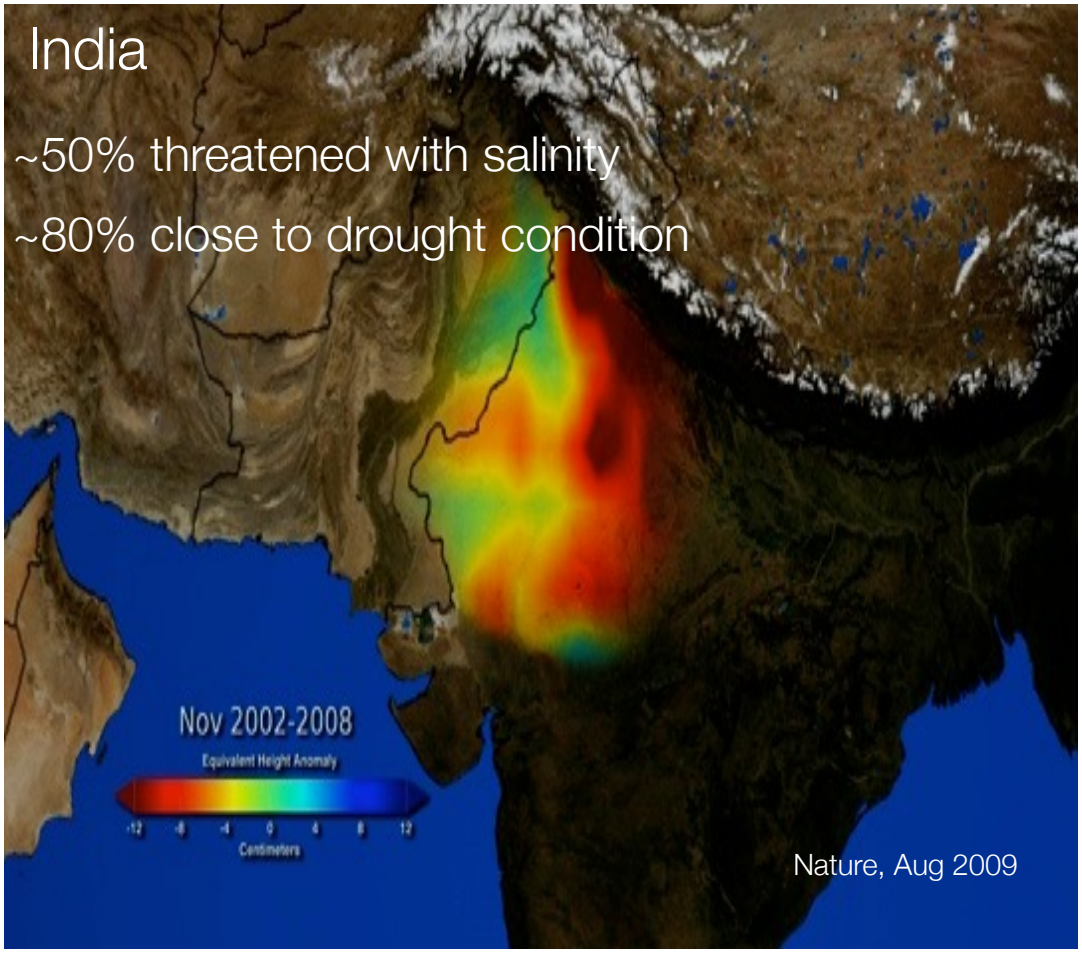
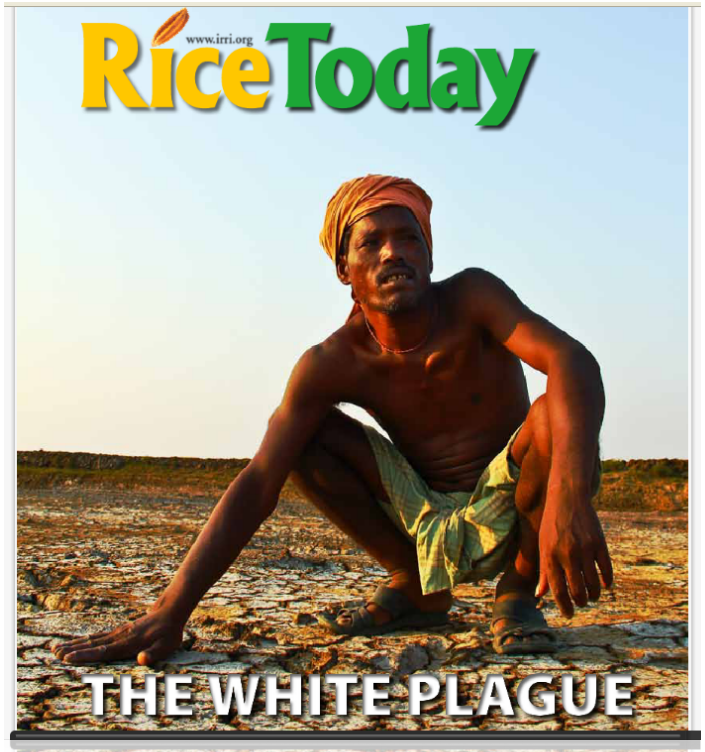
Salinity and drought: two serious threats to agricultural yield

NASA satellites unlock secret to Northern India's vanishing water

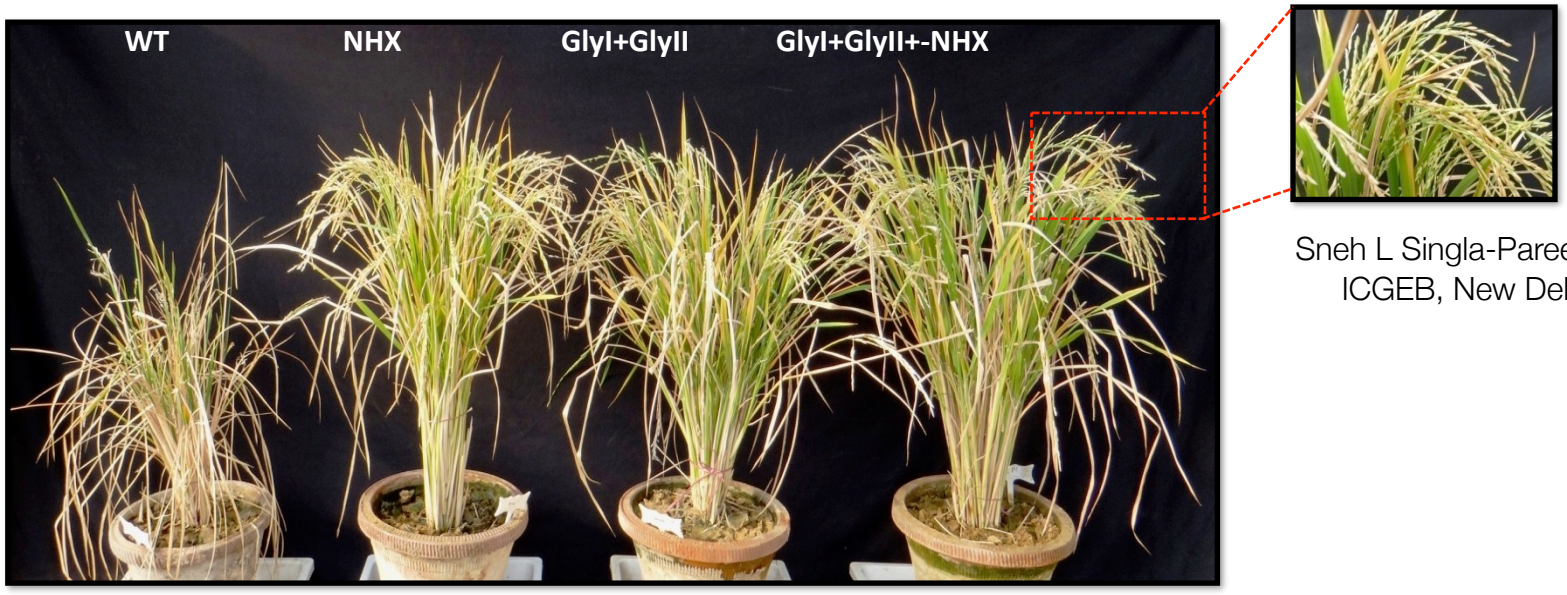


Haryana, Punjab, Rajasthan and Delhi (the grain baskets of India) lost 109 cubic km of ground water in last 6 yrs (2002-08)



Sneh L Singla-Pareek
ICGEB, New Delhi

Triple transgenic rice plants for durable stress tolerance



Triple (Gly+GlyII+NHX) transgenic rice plants show better reproductive growth as compared to double (GlyI+GlyII) or single (NHX1) transgenic lines and WT plants under salinity stress conditions

Millions of hectares of land throughout the world are too saline to produce economic crop yields, and more land becomes nonproductive each year because of salt accumulation. Salinity problems in agriculture affect arid and semiarid regions where rainfall is not sufficient to transport salts from the plant root zone (25% of the earth's surface)

Biological pesticides for pest control

- Understanding mode of action of BT insecticidal proteins.
- Pyramiding of candidate bt genes in transgenes to delay onset of resistance in insects to Bt proteins

- Development and application of Biopesticide

- Biopesticide based on insect symbiont bacteria for agriculture and horticultural crops developed and commercialised, over 300,000 litres sold in 2008

- Analysis of mosquito response to malaria parasite invasion

Search for Novel biomolecules with Potential Insecticidal Activity

- *Helicoverpa armigera* miR-24 is insecticidal
- Feeding of synthetic miR-24 to larvae ceased growth and molting of larvae
- Larvae fed on transgenic plants expressing miR-24 did not molt and died eventually

Buffer-fed larva

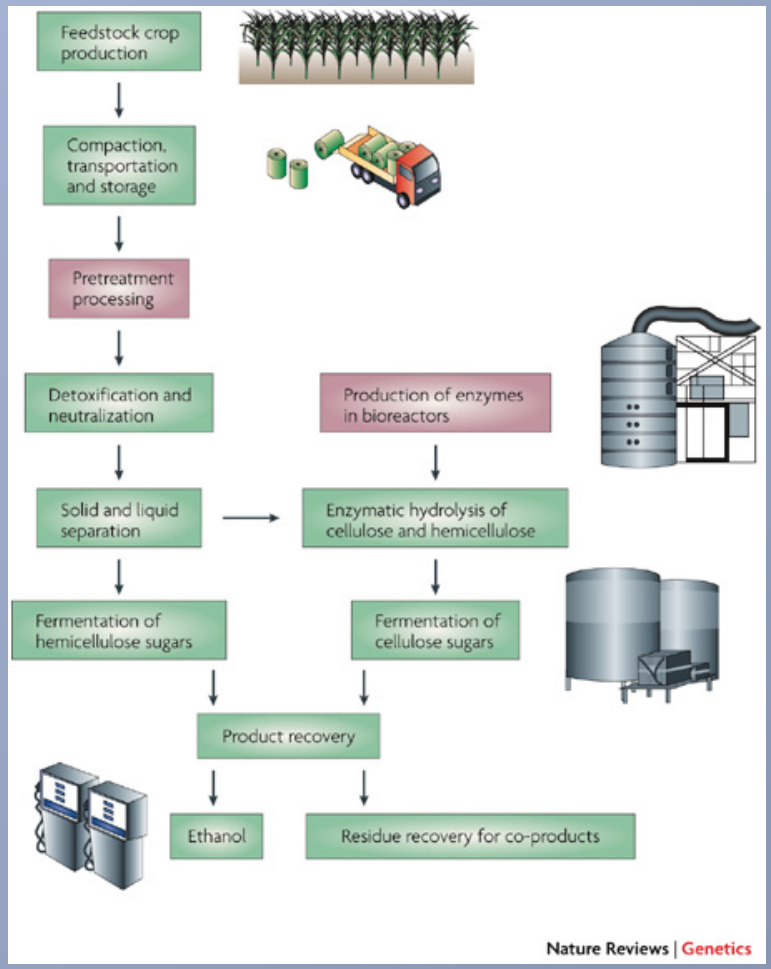
Scrambled miR-24 fed

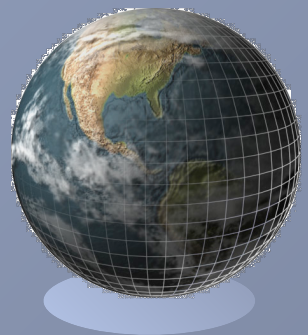
miR-24 fed larva

Genetic engineering for biofuel production

Biofuels provide a potential route to avoiding the global political instability and environmental issues that arise from reliance on petroleum. Currently, most biofuel is in the form of **ethanol** generated **from starch or sugar** (Brazil supplies one quarter of its ground transportation fuel with ethanol from the fermentation of sugarcane sugar), but this can meet only a limited fraction of global fuel requirements. In addition, starch and sugar that are used for for the production of ethanol compete with food supplies.

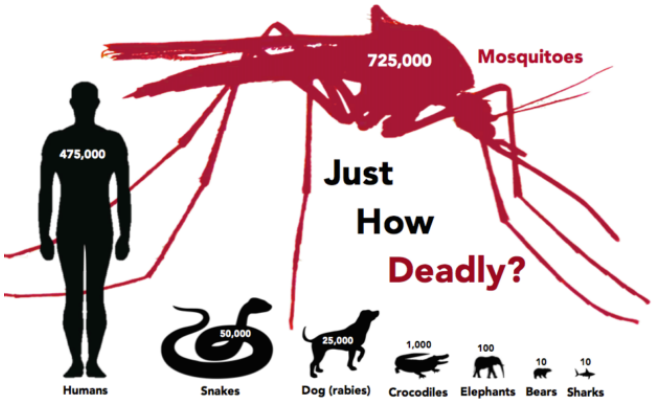
Conversion of **cellulosic biomass**, which is both abundant and renewable, is a promising alternative. However, the cellulases and pretreatment processes involved are very expensive. **Genetically engineering plants to produce cellulases and hemicellulases**, and to reduce the need for pretreatment processes through lignin modification, are promising paths to solving this problem, together with other strategies, such as increasing plant polysaccharide content and overall biomass.

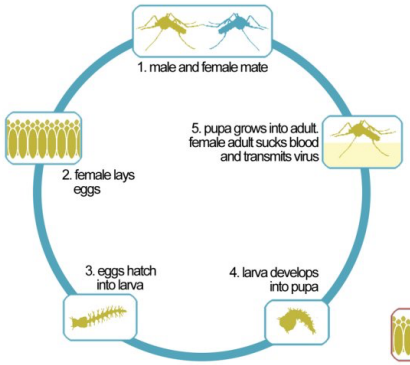




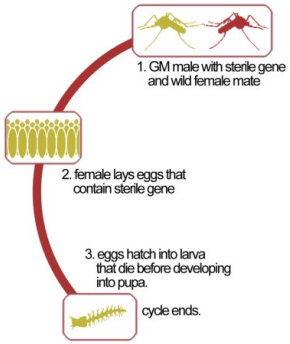
Genetically modified mosquitoes to combat Zika virus

The genetically modified *Aedes aegypti* mosquitoes, created by the British firm Oxitec, are part of an effort to combat the spread of the mosquito-borne Zika virus. The mosquitoes are altered so their offspring die before they are able to reproduce, reducing the population of the *Aedes* mosquito that transmits Zika as well as dengue, yellow fever and chikungunya.



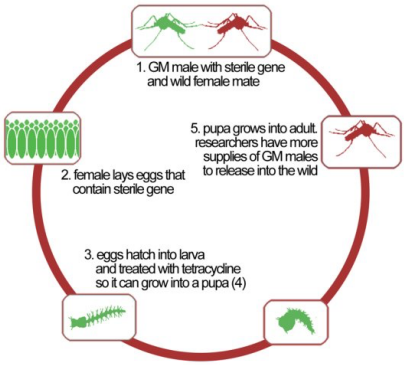


Life cycle of wild male and female mosquitoes produces blood-sucking females

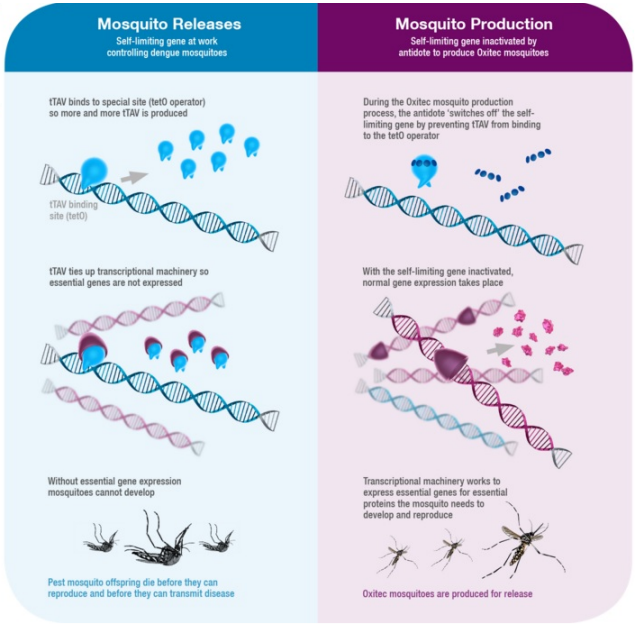


Introduction of GM males breaks this cycle as faulty gene causes offspring to prematurely die

How GM mosquitoes work



More GM males are created in the lab by adding tetracylcine to larvae to allow development



Big companies and GMOs

Mixed Messages

Faced with protests over genetically modified foods, some major food companies are divided over how to deal with the controversy.

GENETICALLY MODIFIED CROPS ARE NOW WIDELY GROWN . .

Soybeans **52%** genetically modified

38 million acres out of 73 million total acres in the United States

Corn **25%** genetically modifi

19 million acres out 76 million total acres in the United States

. . . AND COMPANIES FACE MORE CONFLICTS

◂ NOVARTIS

ONE SIDE Gerber Products, a unit of the company, has banned genetically modified ingredients from its baby-food formulas.

THE OTHER SIDE Novartis is a Swiss pharmaceutical giant that makes and sells genetically modified corn and soybean seeds.

McDONALD'S ▸

ONE SIDE Asked suppliers to stop shipping genetically modified potatoes.

THE OTHER SIDE In its restaurants, French fries are cooked in vegetable oil made from genetically modified corn and soybeans.

◂ PEPSICO

ONE SIDE The Frito-Lay division announced in January that it would stop using genetically modified corn in its chips.

THE OTHER SIDE The company's flagship soft drink uses corn syrup made from genetically modified corn.

The New York Times

Conceptual paper

The Nobel Laureates' Campaign Supporting GMOs

Richard J. Roberts

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A R T I C L E I N F O

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Crop
Pest
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Precision agriculture

A B S T R A C T

More than 800 million people suffer from hunger in the world. Using modern plant breeding methods to generate so-called GMOs (Genetically Modified Organisms), agricultural scientists have shown that crop yields and nutritional quality can be greatly improved. Many GMO varieties have been specifically developed with the aim of being resistant to pests, tolerant to drought and containing beneficial nutrients. This leads to a reduction in the use of insecticides in water and on land. If anything, the GMO varieties are safer than traditionally bred varieties because they are made in a very precise manner. However, the scientific evidence on this issue is being ignored by the Green Parties such as Greenpeace who continue to deny the science and mislead the public. 129 Nobel Laureates have joined in a campaign to convince the Green Parties and the public that they should support the use of GMOs, especially for the sake of the developing world.

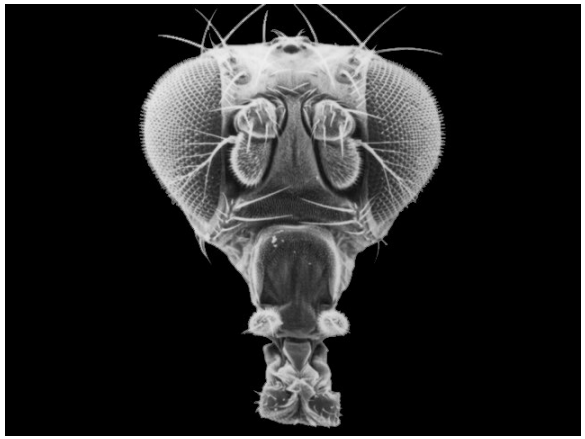
Genetic manipulation of animals to discover gene function

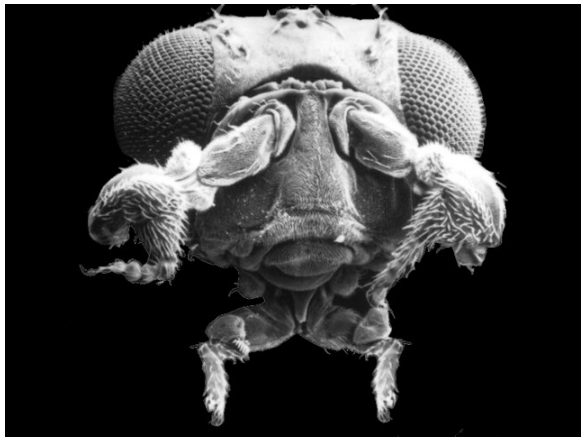
For decades, aspects of physiology and biochemistry have been investigated in animals, and artificial manipulations have often been confined to examining the effect of altering the animal's environment or some aspect of its phenotype

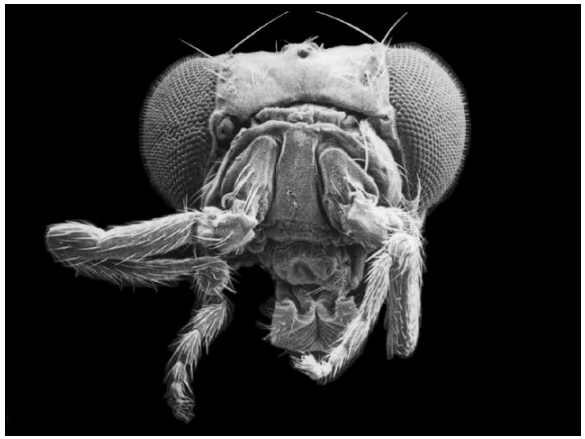
Some animals, notably *Drosophila* and mice, have been particularly amenable to genetic analyses and traditional genetic manipulation of animals has involved carefully selected breeding experiments or exposure of animals to powerful chemical or radioisotopic mutagens (high doses of X rays)

Drosophila melanogaster

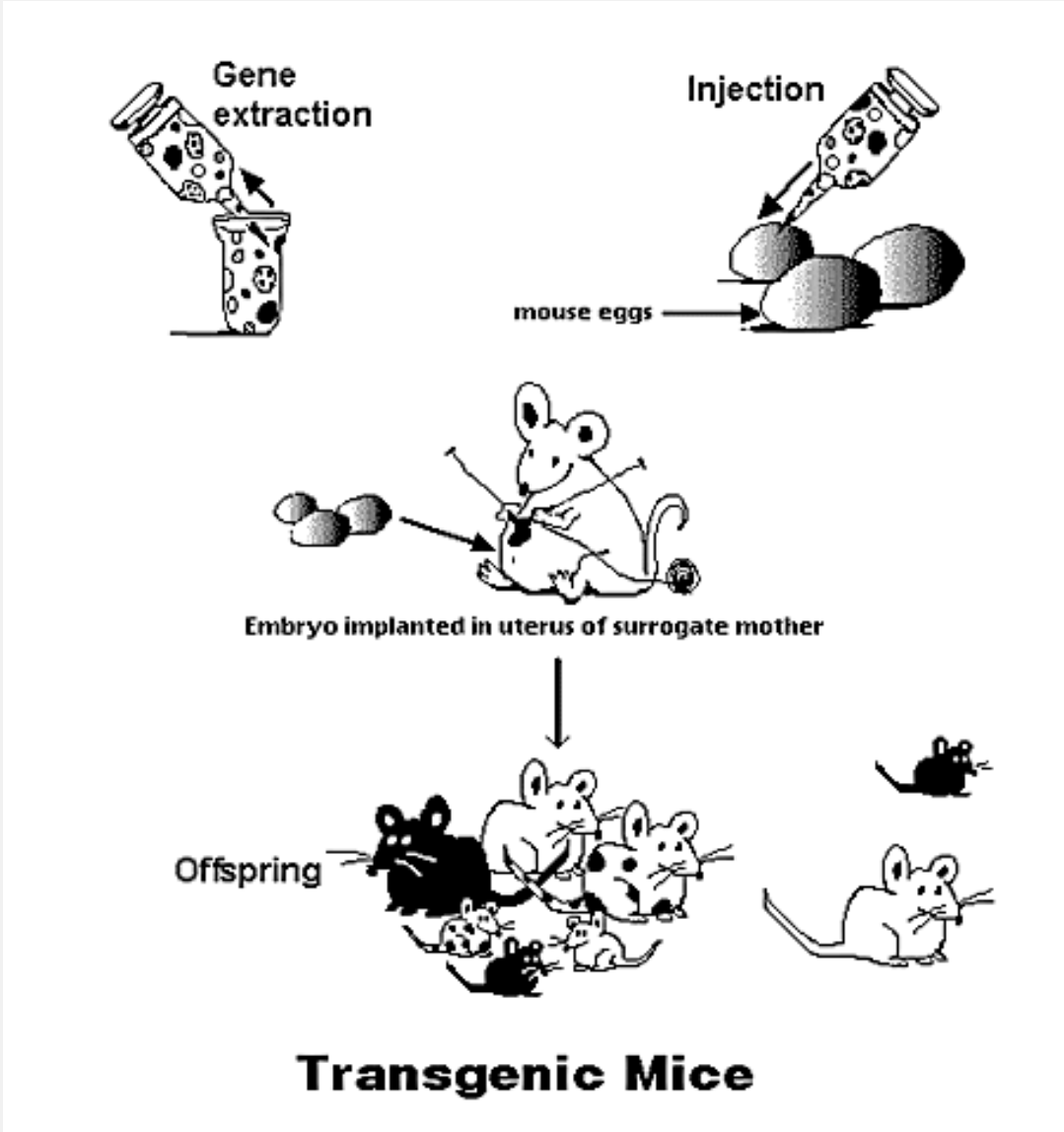
When flies have a mutation wherein the *Antennapedia* gene is expressed in the head (as well as in the thorax), legs rather than antennae grow out of the head sockets







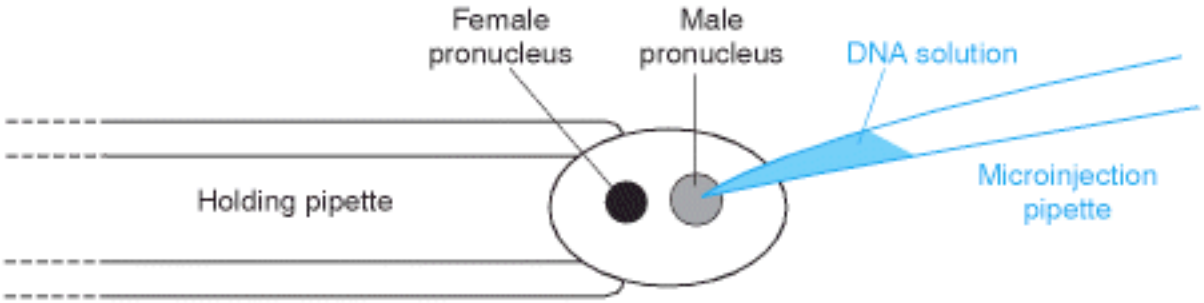
How to construct a transgenic mouse



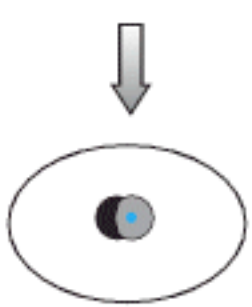
Transgenesis involves transfer of foreign DNA into totipotent or pluripotent embryo cells (either **fertilized oocytes**, **cells of the very early embryo** or **cultured embryonic stem cells**) followed by insertion of the transferred DNA into host chromosomes

Pronuclear microinjection

To obtain transgenic mice, females are superovulated, mated to fertile males and sacrificed the next day. Fertilized oocytes are recovered from excised oviducts. The DNA of interest is then microinjected using a micromanipulator into the male pronucleus of individual oocytes

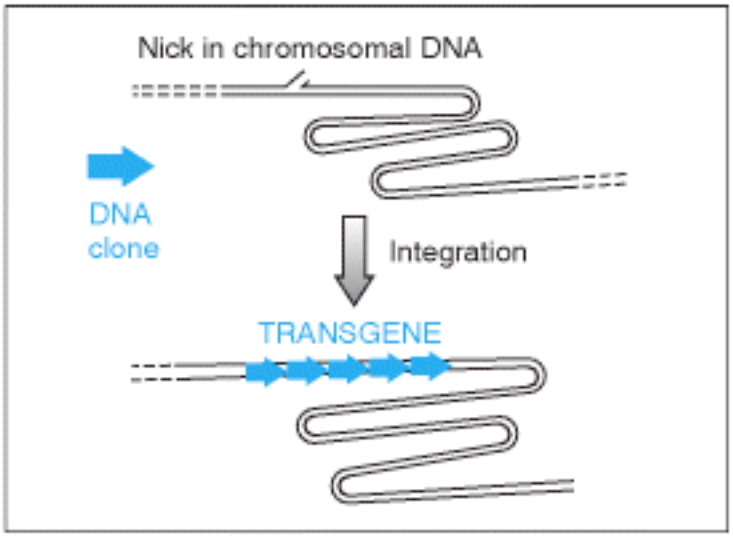


Surviving oocytes are reimplanted into the oviducts of foster females and allowed to develop into mature animals



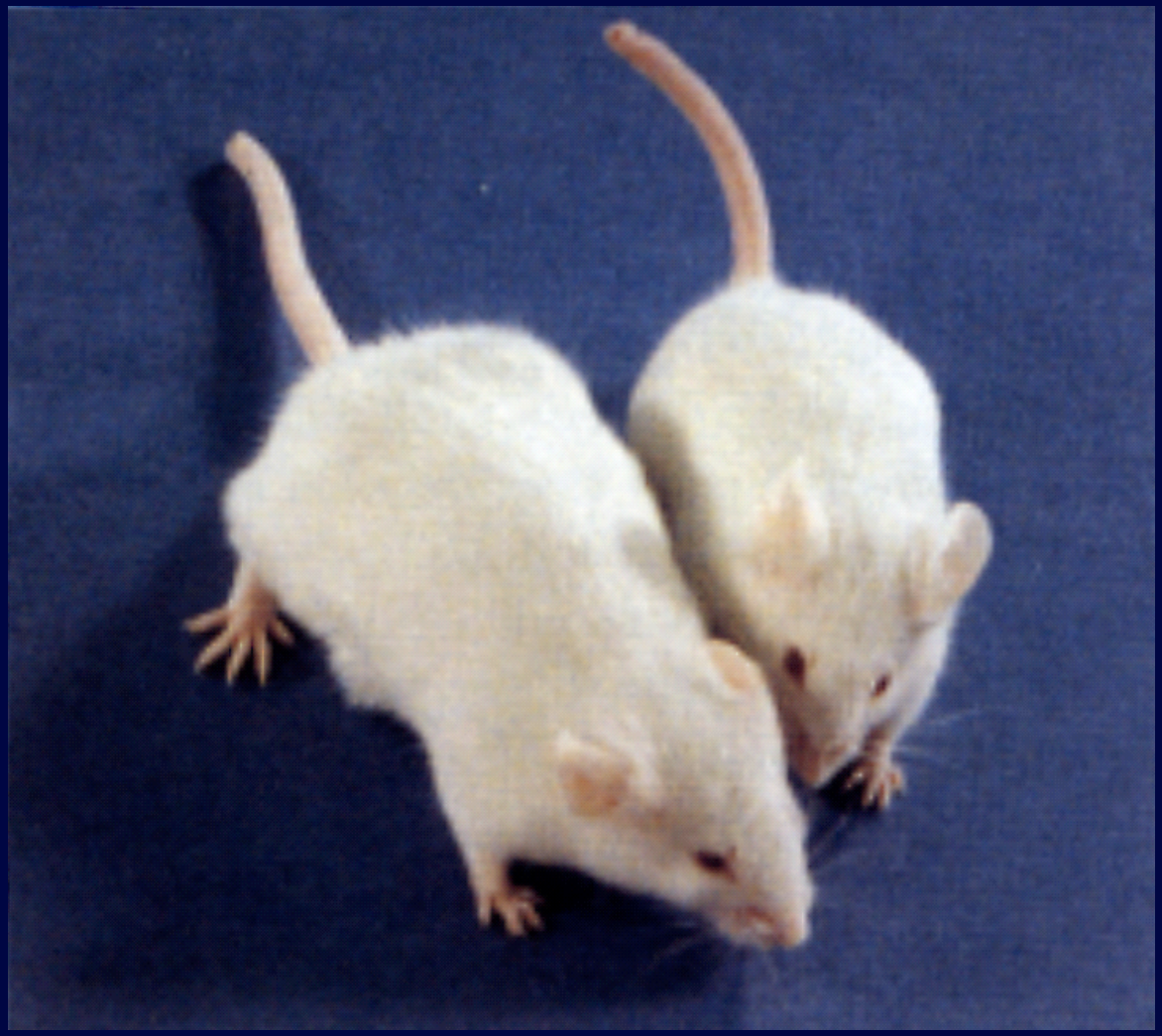
Transfer to oviducts of pseudopregnant female

Transgenic mouse (transgene present in all nucleated cells)

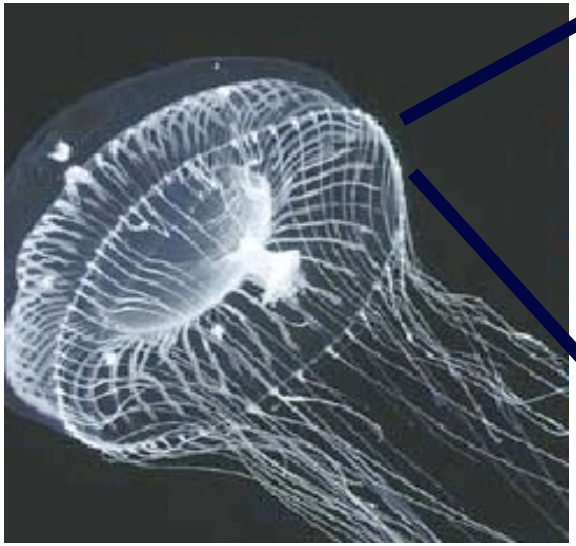


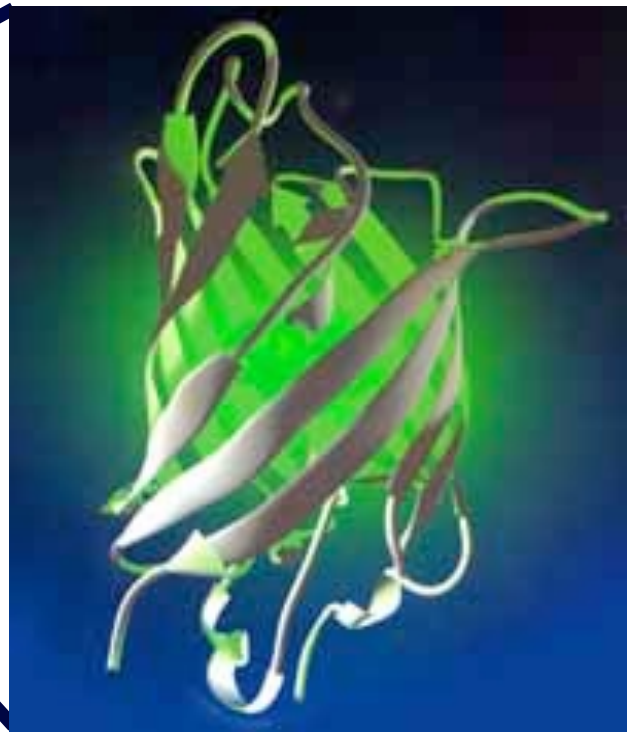
The transgene randomly integrates into chromosomal DNA, usually at a single site, with multiple copies of the transgenes as head-to-tail concatemers (up to more than 50 copies). As a result of chromosomal integration, the transgenes can be passed on to subsequent generations in mendelian fashion

Transgenic mouse for growth hormone

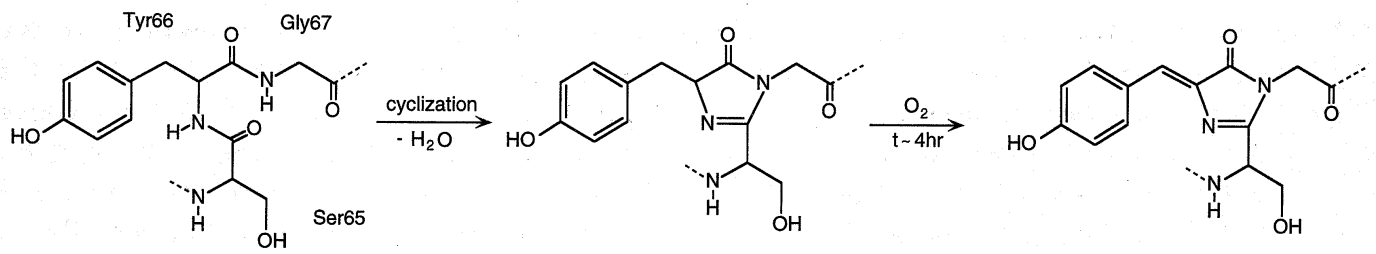


Green fluorescent protein

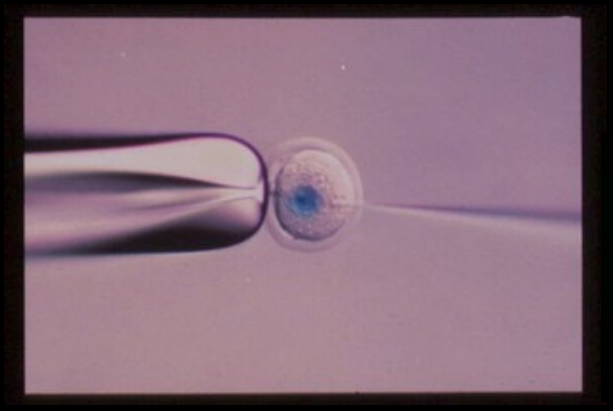




238-amino-acids 27-kD protein containing a photoexcitable greenish-light-emitting chromophore



The GFP transgenic mouse



embryo

The Green Fluorescent Protein (GFP)
from the Aequora victoria jellyfish

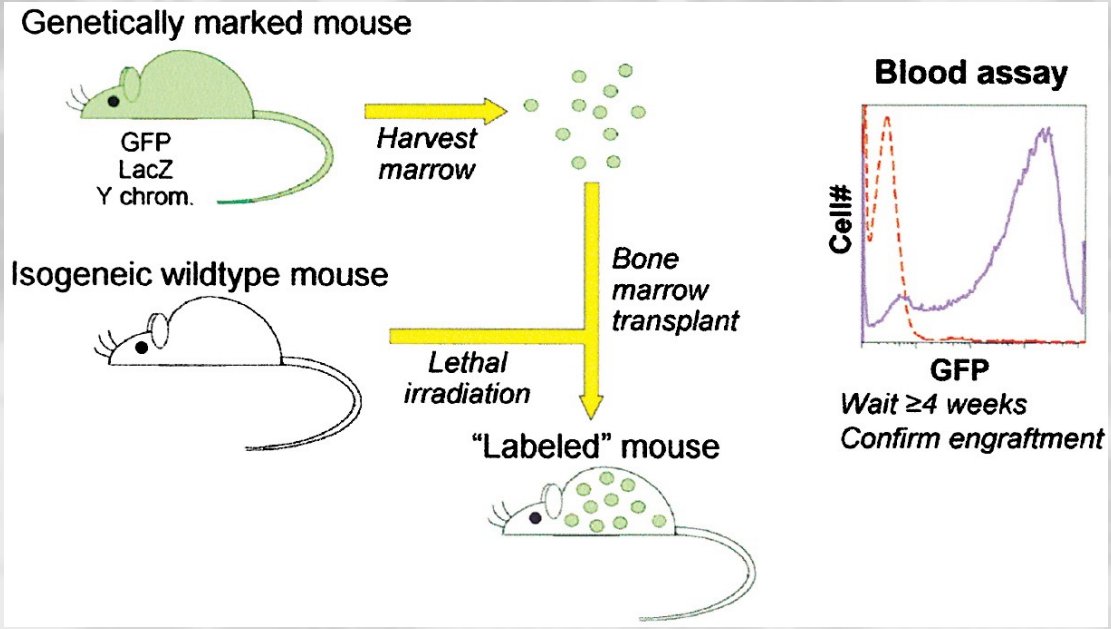
How to study stem cell plasticity

I. *In vivo marking*

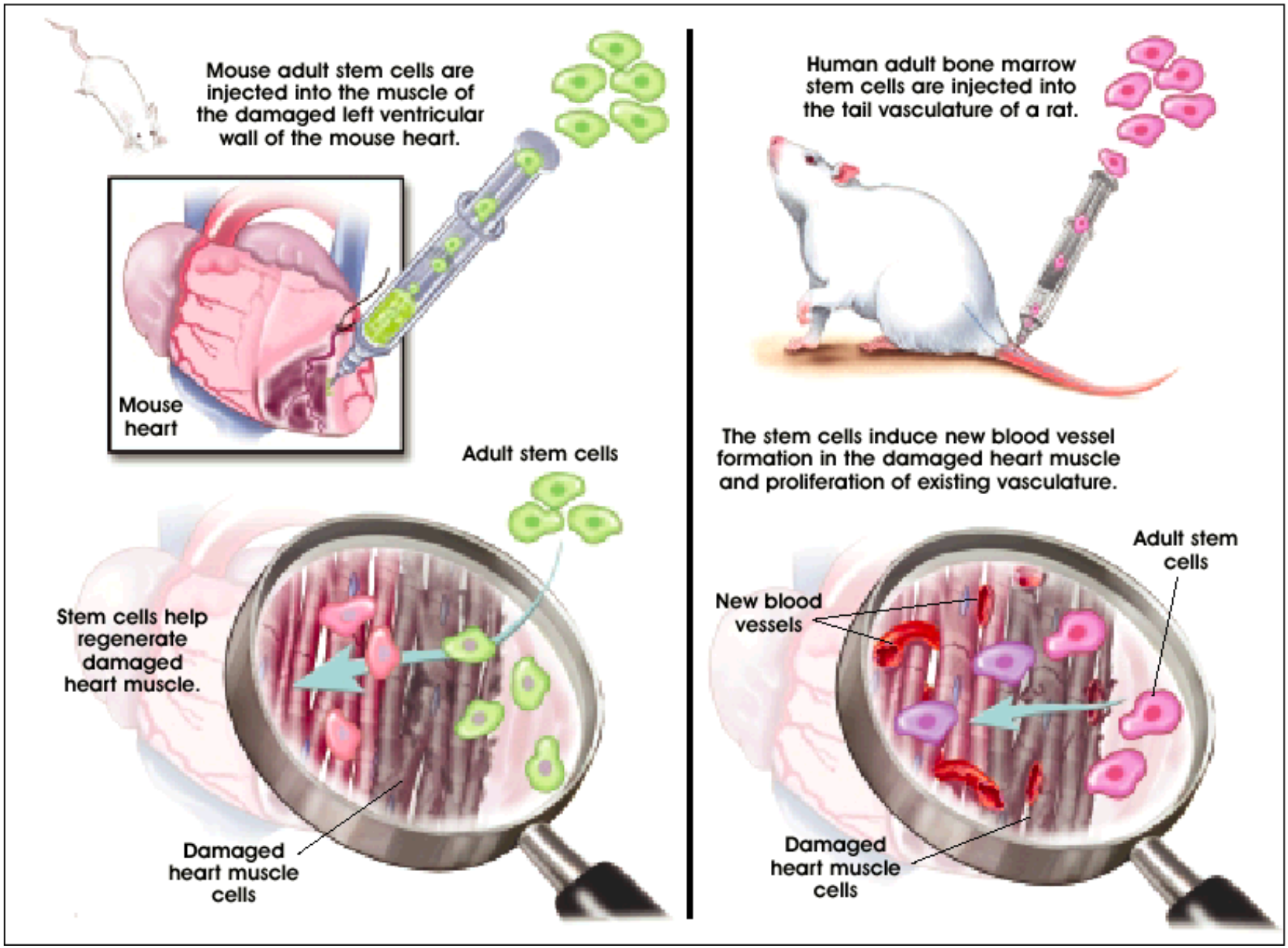
II. *Ex vivo marking: stem cell isolation, marking and transplantation in the same or in an other organism*

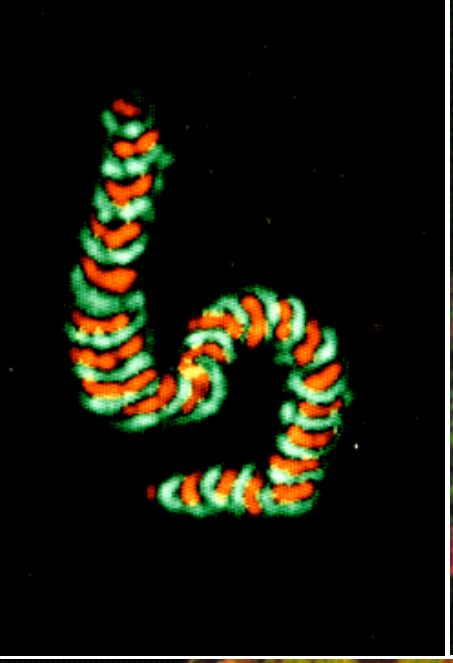
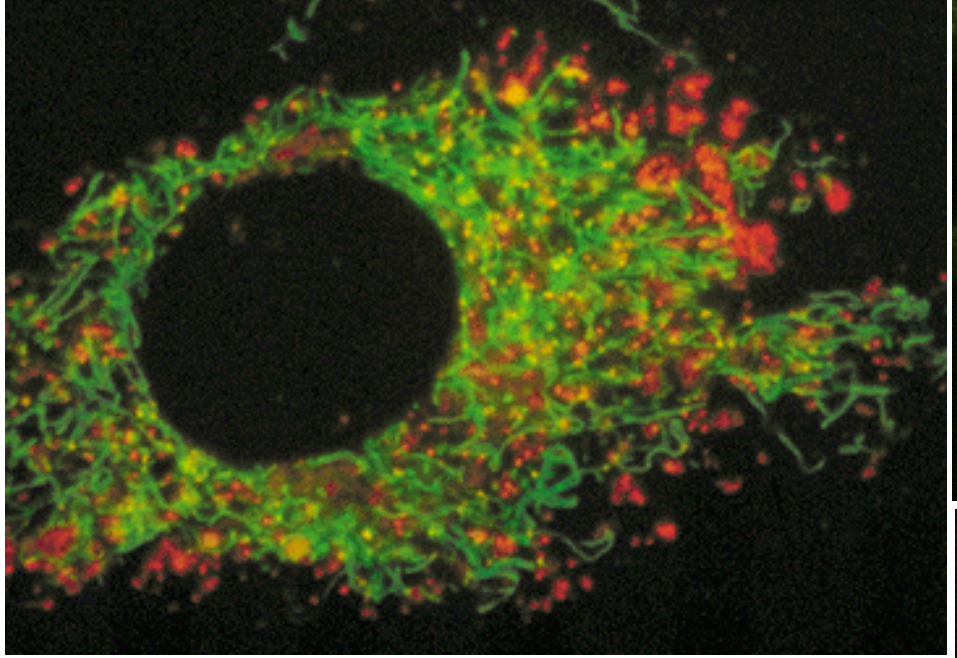
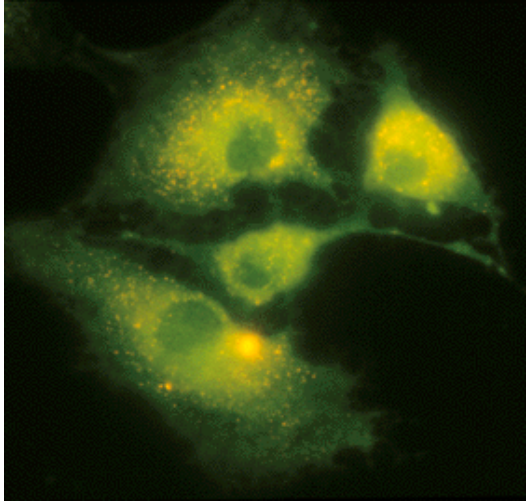
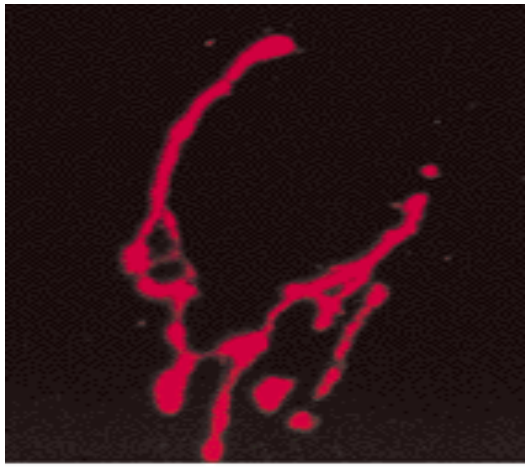
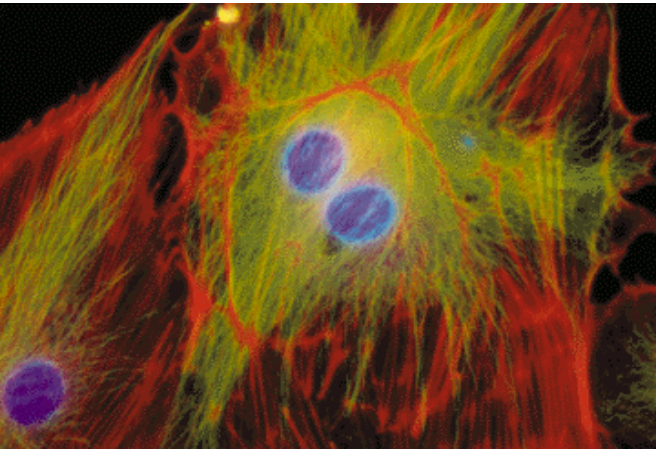
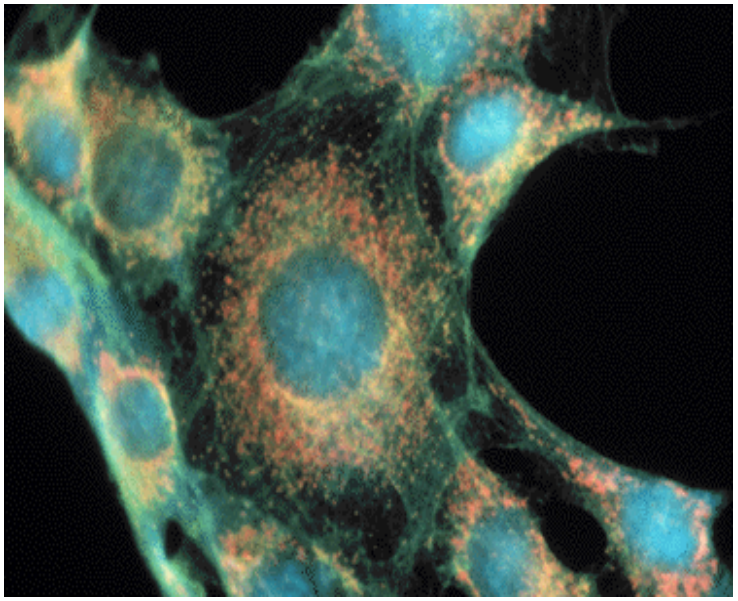
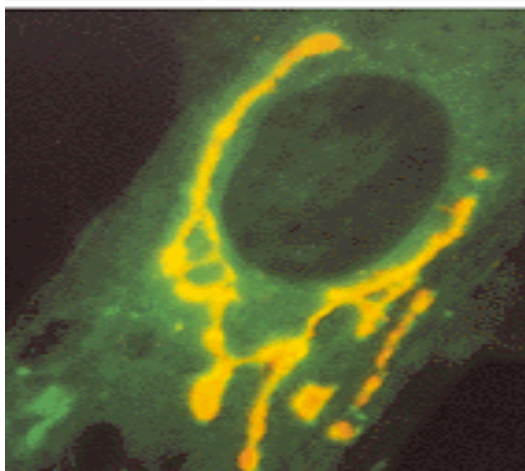
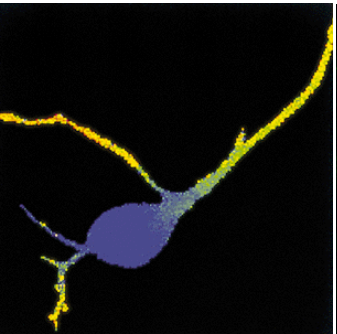
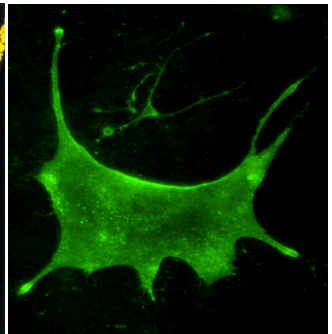
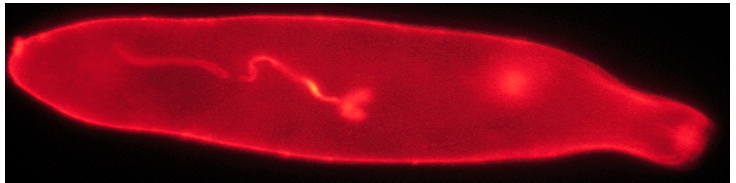
III. *In vitro manipulation (growth factors administration, gene transfer)*

IV. *Stem cells transplantation*

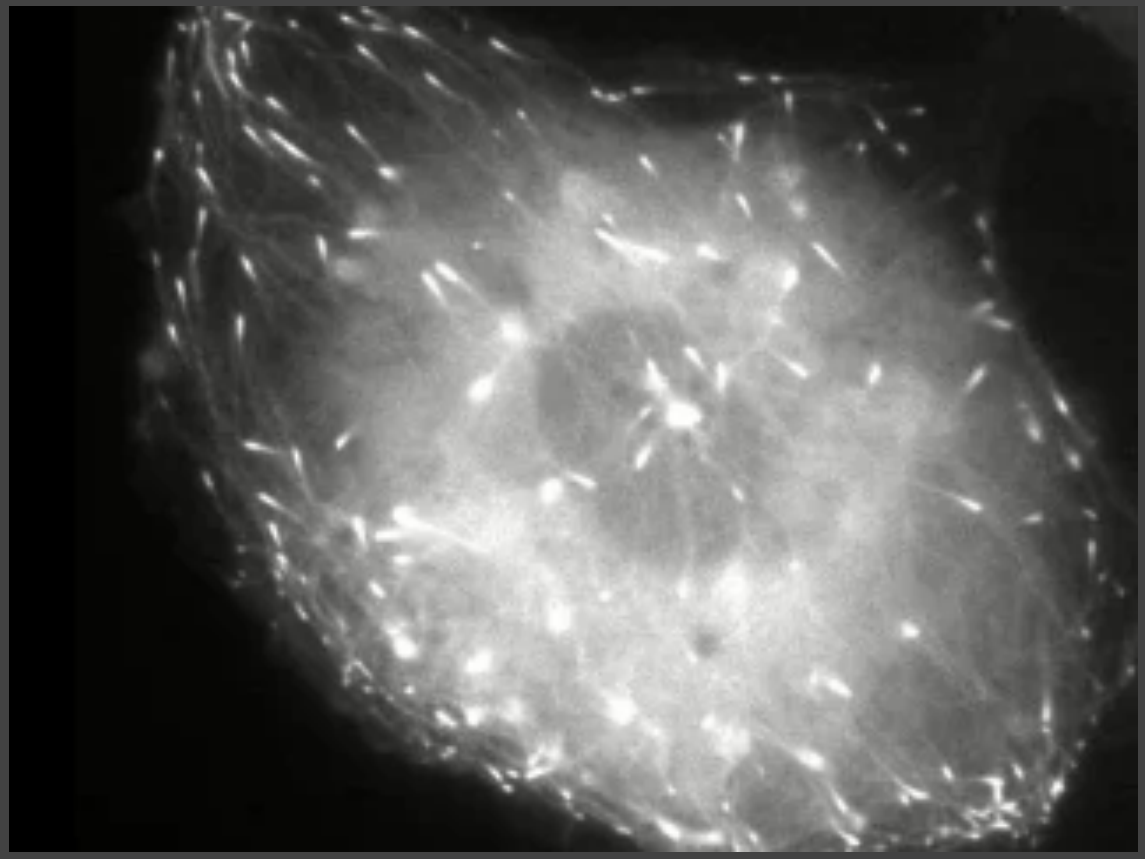


Il midollo osseo per la terapia cellulare dell'infarto cardiaco

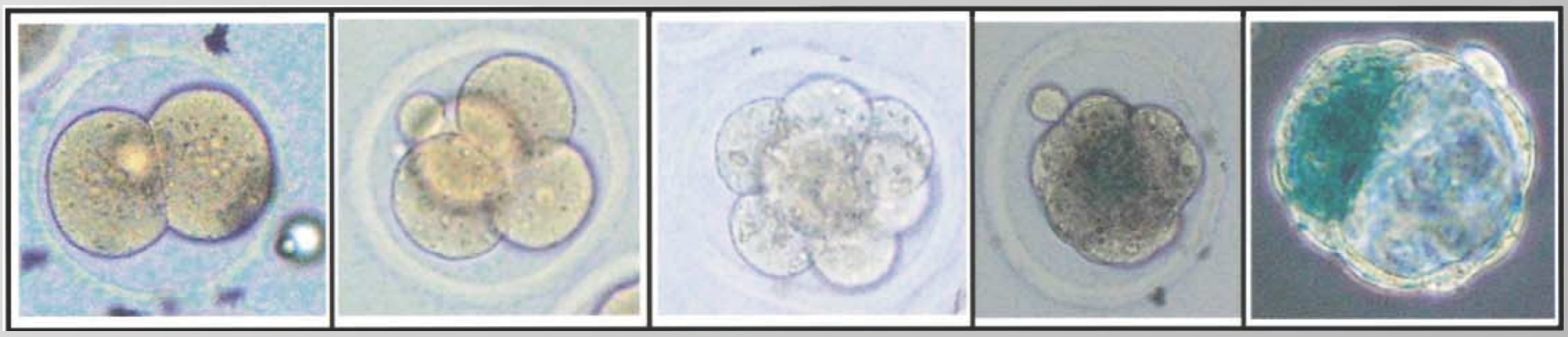


GFP fusion protein to study the cytoskeleton and cell movement



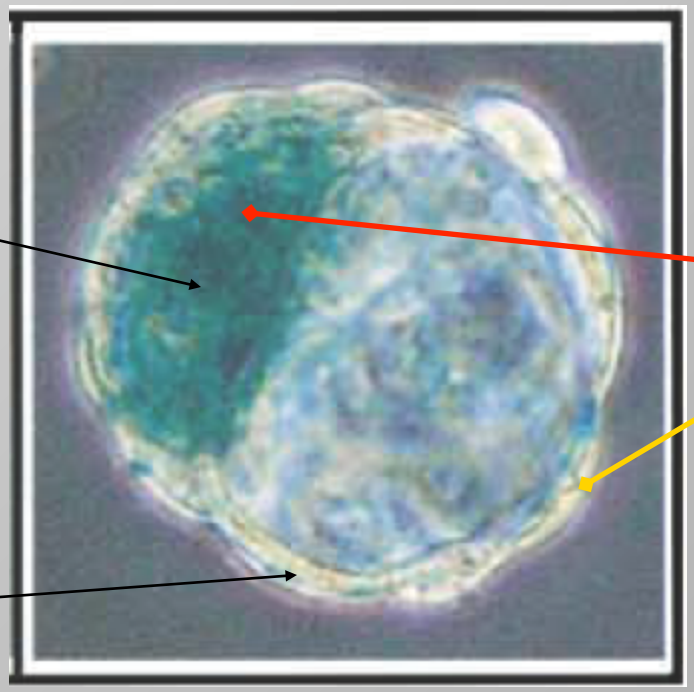
Transgenic animals can be obtained by genetically modifying embryonic stem cells

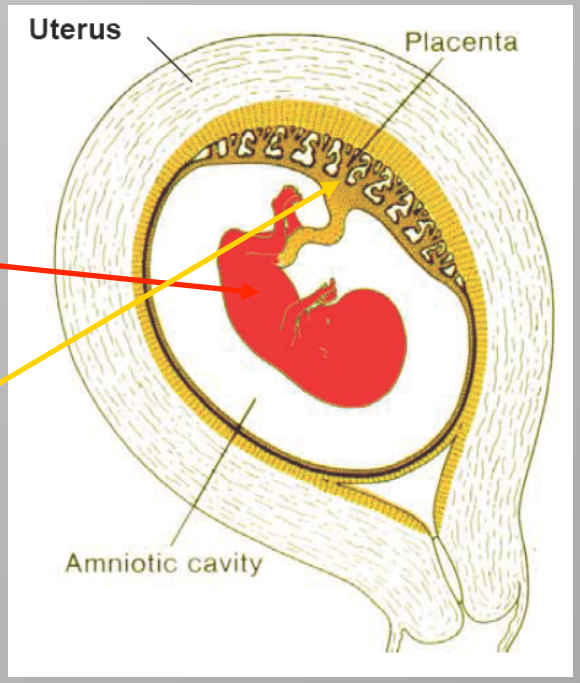


2 cells 4 cells 8 cells morula blastocyst

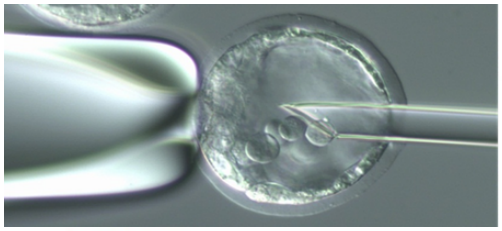
Inner cell mass (ICM)

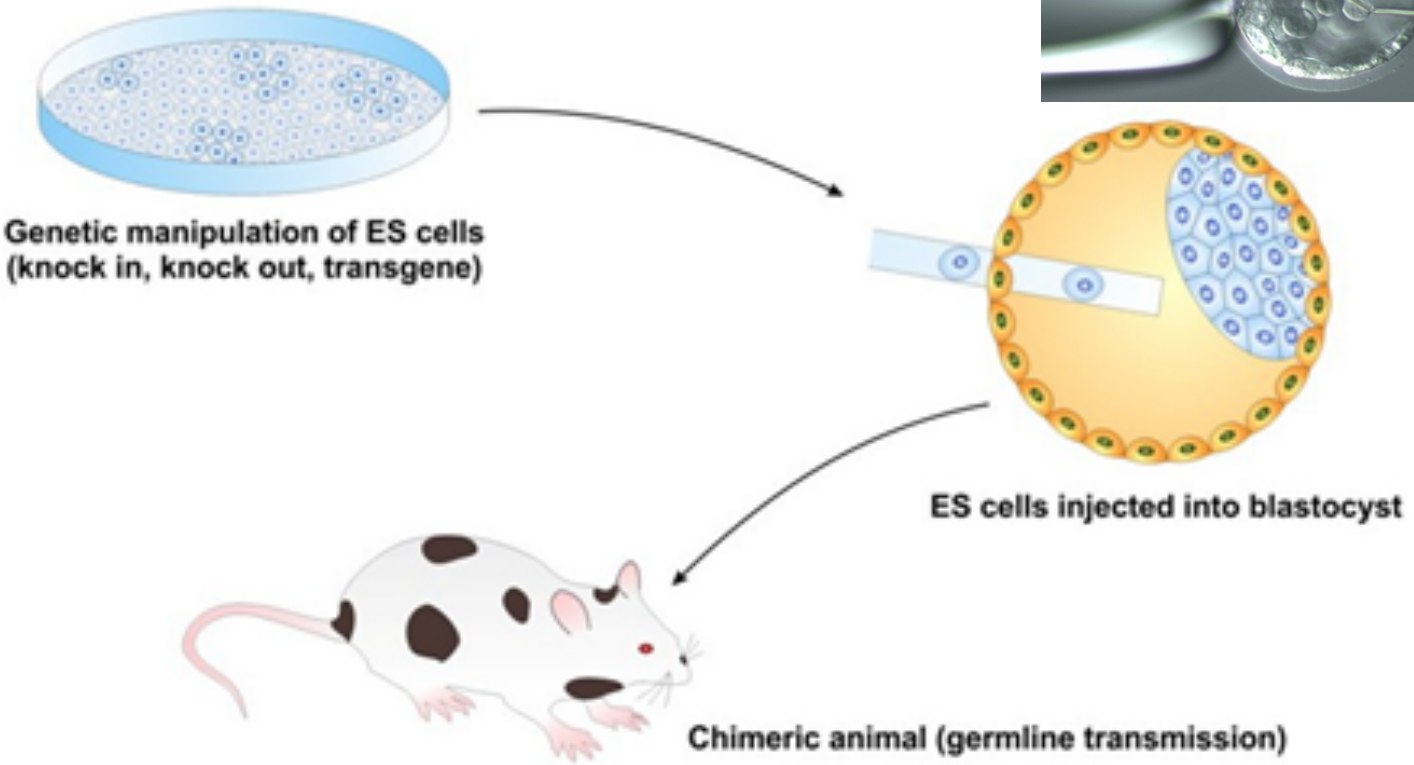
trophectoderm





A simpler alternative, suitable to large-scale production of transgenic animals (only mice) involves transferring the foreign DNA into cultured embryonic stem (ES) cells, which can be cultured and retain the potential to contribute to all of the tissues of a mouse when injected back into a host blastocyst and reimplanted in a pseudopregnant mouse

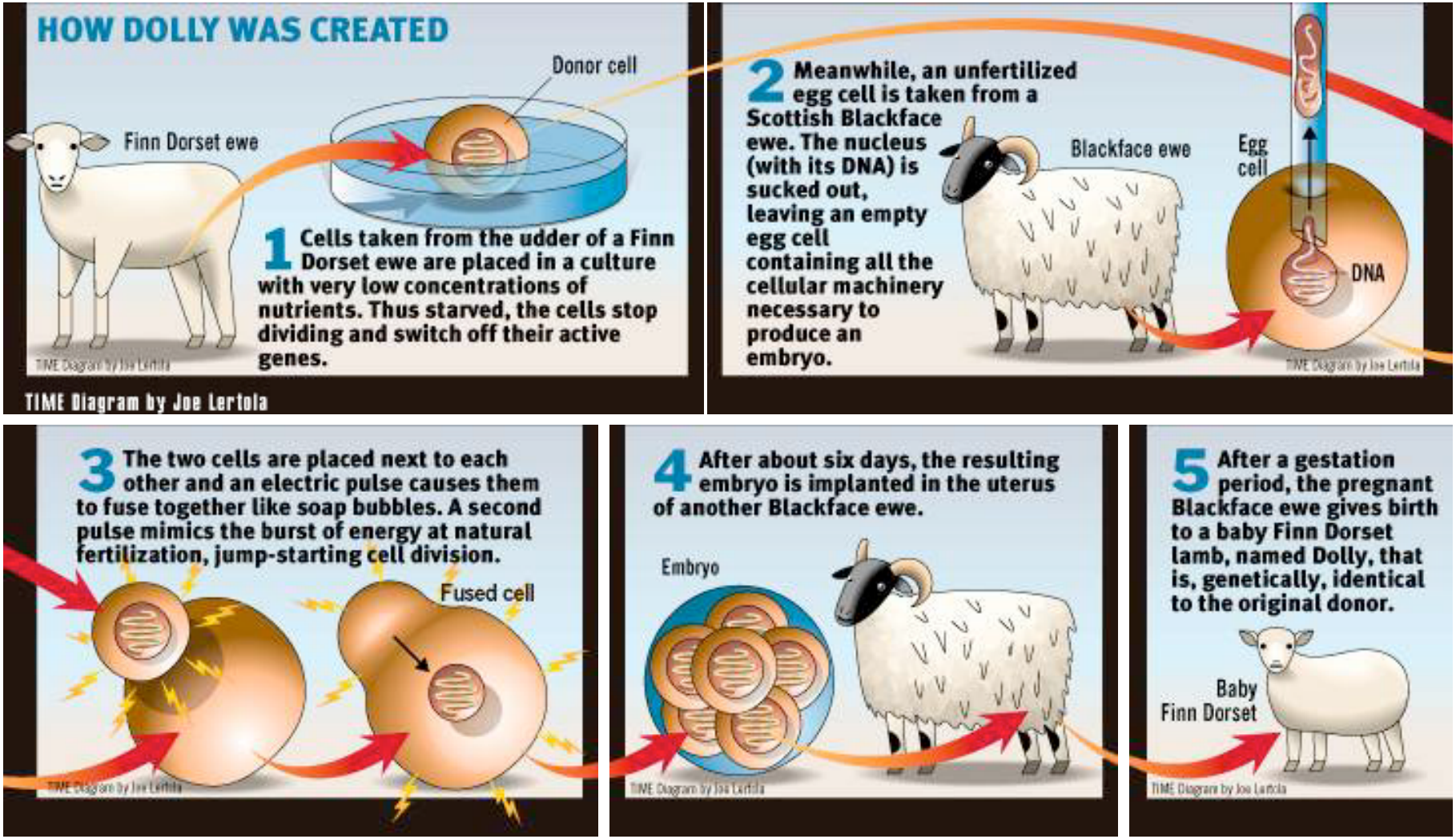




The developing embryo is a chimera, containing cells derived from the blastocyst and the implanted ES cells. If the two strains of cells are derived from mice with different coat colors, chimeric offspring can easily be identified

Use of genetically modified ES cells results in a partially transgenic mouse. Fully transgenic mice are usually obtained by screening the offspring of matings between chimeras (usually males) and mice with a coat color recessive to that of the strain from which the ES cells were derived

Animal cloning by nuclear transfer



Evidence of a Pluripotent Human Embryonic Stem Cell Line Derived from a Cloned Blastocyst

Woo Suk Hwang,[1,2]* Young June Ryu,[1] Jong Hyuk Park,[3] Eul Soon Park,[1] Eu Gene Lee,[1] Ja Min Koo,[4] Hyun Yong Jeon,[1] Byeong Chun Lee,[1] Sung Keun Kang,[1] Sun Jong Kim,[3] Curie Ahn,[5] Jung Hye Hwang,[6] Ky Young Park,[7] Jose B. Cibelli,[8] Shin Yong Moon[5]*

Somatic cell nuclear transfer (SCNT) technology has recently been used to generate animals with a common genetic composition. In this study, we report the derivation of a pluripotent embryonic stem (ES) cell line (SCNT-hES-1) from a cloned human blastocyst. The SCNT-hES-1 cells displayed typical ES cell morphology and cell surface markers and were capable of differentiating into embryoid bodies in vitro and of forming teratomas in vivo containing cell derivatives from all three embryonic germ layers in severe combined immunodeficient mice. After continuous proliferation for more than 70 passages, SCNT-hES-1 cells maintained normal karyotypes and were genetically identical to the somatic nuclear donor cells. Although we cannot completely exclude the possibility that the cells had a parthenogenetic origin, imprinting analyses support a SCNT origin of the derived human ES cells.

[1]College of Veterinary Medicine, [2]School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea. [3]Medical Research Center, MizMedi Hospital, Seoul, 135-280, Korea. [4]Gachon Medical School, Incheon, 417-840, Korea. [5]College of Medicine, Seoul National University, Seoul, 110-744, Korea. [6]School of Medicine, Hanyang University, Seoul, 471-701, Korea. [7]College of Natural Science, Sunchon National University, Sunchon, 540-742, Korea. [8]Department of Animal Science-Physiology, Michigan State University, East Lansing, MI 48824, USA.

Woo-Suk Hwang
& the problem of research misconduct

"He was a national hero in South Korea, his research lab was probably one of the best funded in the world, and he flew first class anywhere he wanted, any time he wanted, for free, courtesy of Korean Air. He was treated like a rock star. His spectacular fall from one of the most envied positions in science plays out like a Greek tragedy."

Stephen Minger: *The Fall of a Scientific "Rock Star"*. BBC online: Tuesday, 10 January 2006, 17:53 GMT. http://news.bbc.co.uk/1/hi/sci/tech/4599974.stm

Originally published in *Science* Express on 12 February 2004
Science 12 March 2004:
Vol. 303. no. 5664, pp. 1669 - 1674
DOI: 10.1126/science.1094515

REPORTS

This article has been retracted

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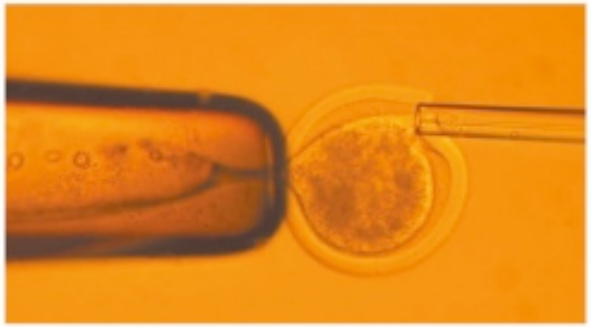
THE RISE AND FALL AND RISE OF WOO SUK HWANG



FEBRUARY 2004
Woo Suk Hwang describes the first stem-cell line, NT-1, derived from a cloned human embryo.

MAY 2005
Hwang's group publishes a second paper reporting 11 further human embryonic cell lines.

AUGUST 2005
Hwang's group is the first to clone a dog.

NOVEMBER 2005
US collaborator Gerald Schatten splits with Hwang, citing ethical problems in getting human eggs.

DECEMBER 2005
Pushed by increasing evidence, Seoul National University (SNU) launches an investigation.

JANUARY 2006
Hwang's human-cloning research is deemed fraudulent by SNU. His dog-cloning claims are upheld.

JULY 2006
Sooam Foundation starts up, with US$3.5 million from Hwang's supporters.

2007
The Korean health ministry grants Sooam the right to do human-embryo and cloning research.

OCTOBER 2009
Hwang is found guilty of embezzlement and bioethics violations. Appeal continues.

2011
Canada grants Hwang a patent for the NT-1 cell line.

2012
Sooam scientists clone a coyote using a dog egg-cell donor and surrogate mother.

2013
Court tells the Korean Centers for Disease Control and Prevention to register the NT-1 cell line.

Woo-Suk Hwang, cloning of Snuppy

Hwang WS, *et al.* (2005). "Dogs cloned from adult somatic cells". *Nature* **436** (7051): 641. PMID 16079832 DOI:10.1038/436641a.

theguardian

SHORTCUTS
BLOG
A SIDEWAYS LOOK AT THE NEWS

If your dog is about to die, why not clone it?

A researcher in South Korea claims he can clone your pet. All he needs is some tissue from the animal and £66,000

Spot the difference ... 'The spots on a dalmatian clone will be different from the original.' Photograph: Alamy

Insung Hwang's business, according to his website, is "healing broken hearts". Specifically those of people who have lost a beloved dog. Now he is to offer his therapeutic services in the UK.

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www.continental.com
www.state.gov

Trakr, il cane eroe dell'11 settembre

Stati Uniti

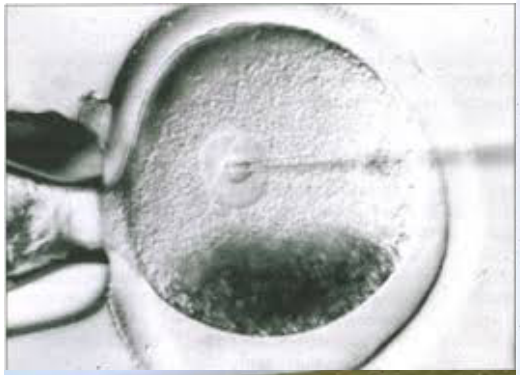
Cinque cloni di Trakr, il cane-eroe trovò l'ultimo superstite dell'11/9

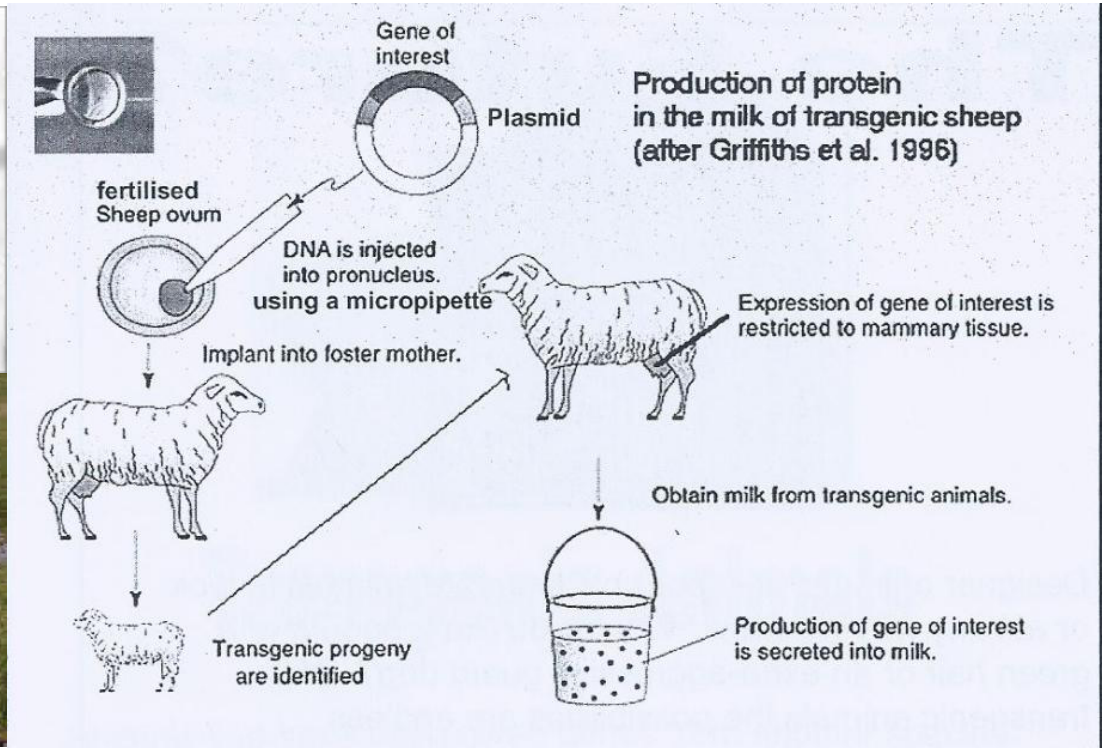
WASHINGTON—Trakr, il cane-eroe chel'11 settembre trovò l'ultimo sopravvissuto a Ground Zero, è stato clonato. A eseguire l'operazione, in Corea del Sud, è stato il controverso professore Hwang Woo-Suk, che nel 2005 clonò il primo cane. È stato lo stesso padrone di Trakr a volere che il suo cane, morto due mesi fa, venisse clonato. E ora cinque piccole "copie" dell'eroe canino potranno essere comprate per 100 mila dollari.

Move over Dolly, and make room for Polly

Polly was created by the same team at the Roslin Institute that gained fame earlier this year with the birth of Dolly, the first sheep cloned using adult animal cells.

The Edinburgh company that sponsors the research, PPL, already produces transgenic sheep that produce alpha-1-antitrypsin, a protein used to treat the symptoms of cystic fibrosis. Transgenic sheep have also been genetically engineered to produce a milk containing proteins used by patients with clotting disorders such as hemophilia, including fibrinogen, factor VII and factor IX.



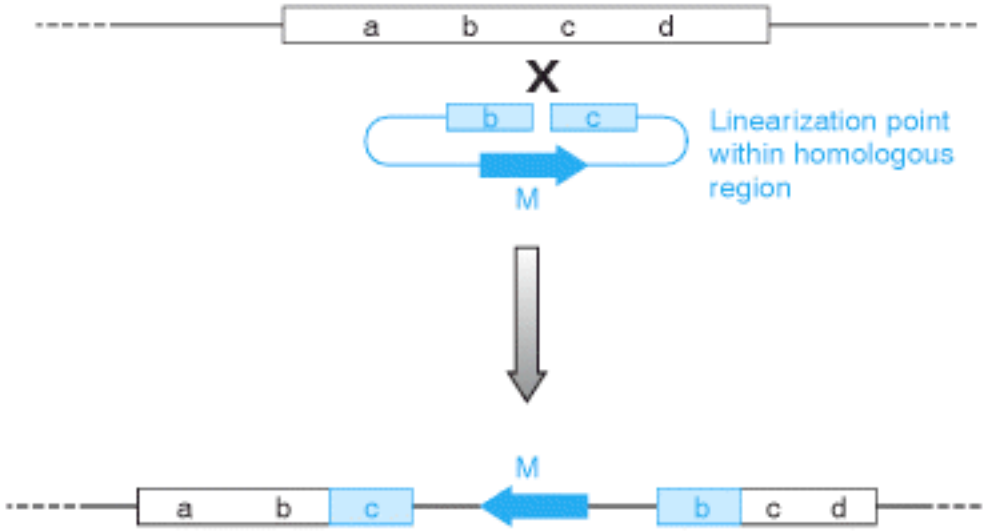


Gene targeting by homologous recombination in ES cells can be used to produce mice with a mutation in a predetermined gene

Gene targeting typically involves introducing a mutation by homologous recombination in mouse ES cells: once a mutation has been engineered into a specific mouse gene within the ES cells, the modified ES cells can then be injected into the blastocyst of a foster mother and eventually a mouse can be produced with the mutation in the desired gene in all nucleated cells

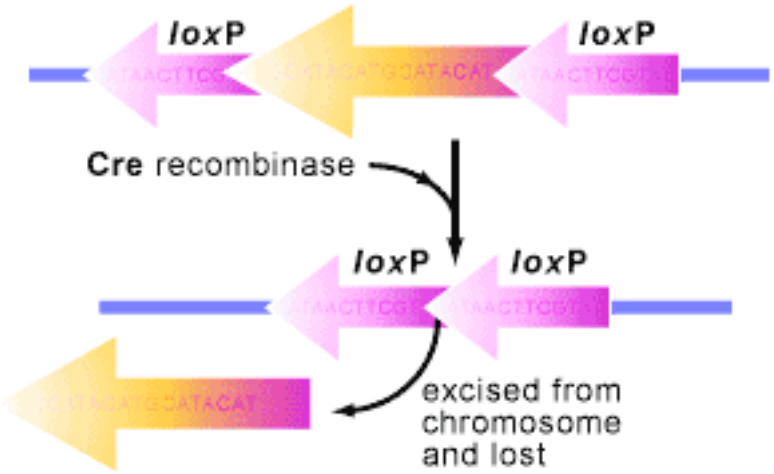
Homologous recombination in mammalian cells is a very rare occurrence and its frequency is increased when the degree of sequence homology between the introduced DNA and the target gene is very high

To assist identification of the desired homologous recombination events, the targeting vector (often a plasmid vector) contains a marker gene, such as the *neo* gene, which permits selection for cells that have taken up the introduced DNA.



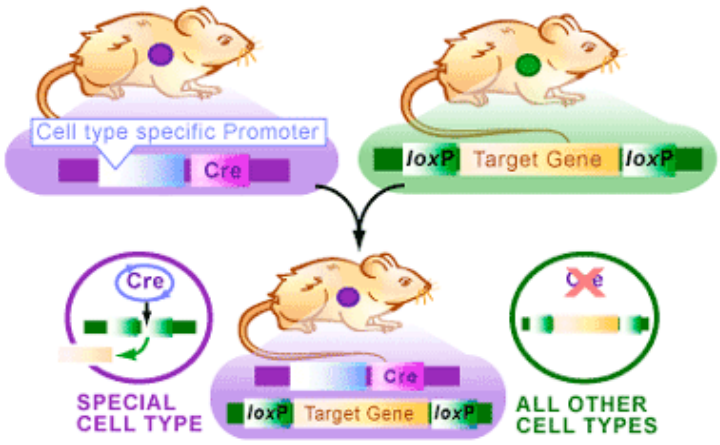
The gene in question is selectively inactivated, producing a **'knock-out' mouse**, and the effect of the mutation on the development of the mouse is monitored

The Cre-loxP system extends the power of gene targeting, by allowing site-specific recombination



The *lox*P sequence consists of 34 bp and comprises two inverted 13 bp repeats separated by a central asymmetric 8 bp spacer

Cre, short for **c**yclization **re**combination, is a site-specific DNA recombinase, which can recombine DNA when it locates specific sites in a DNA molecule. These sites are known as ***loxP*** sequences, which are 34 base pairs long and magnets for the Cre to recombine the DNA surrounding them.

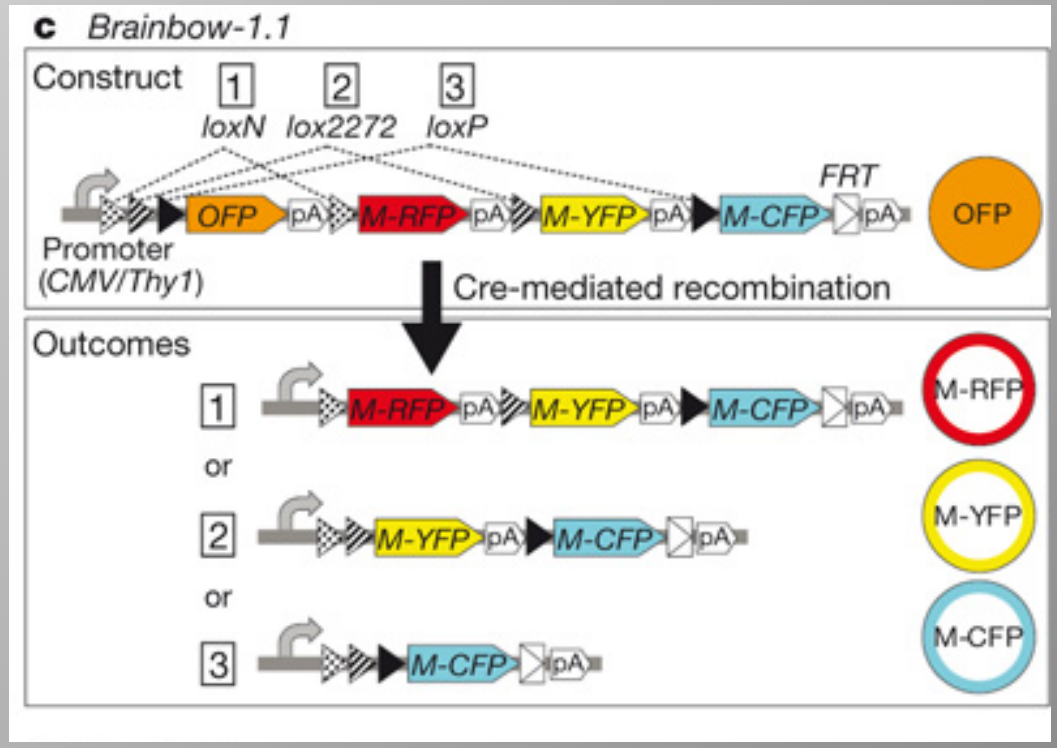


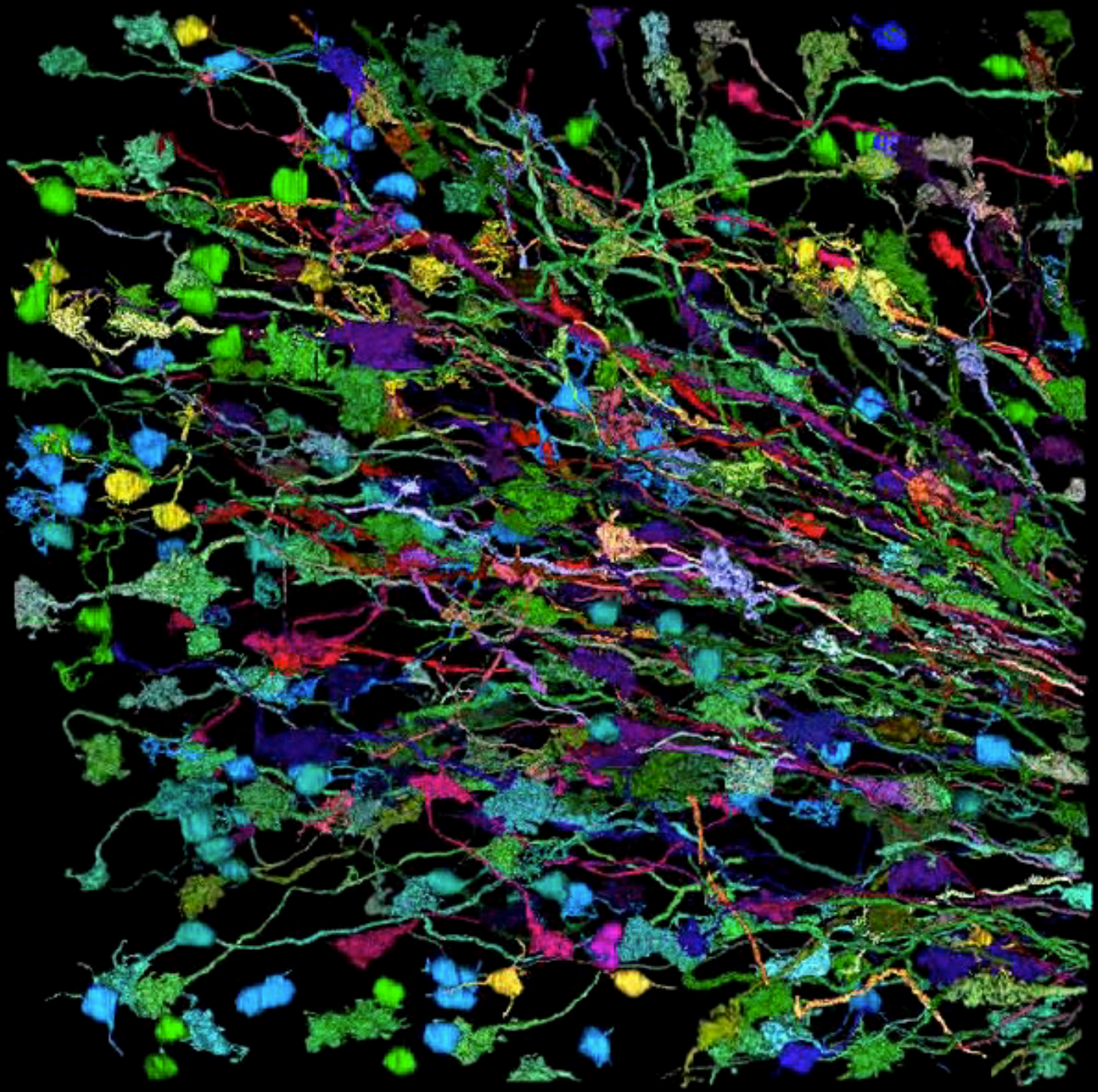
For genes that are vital to early development simple knock-out experiments are not helpful because death ensues at the early embryonic stage. The **conditional knock-out** has been developed to inactivate expression of the target gene in only selected, predetermined cells of the animal

The animal can therefore survive and the effect of the knock-out can be studied in a tissue or cell type of interest.

"Having cells go where they're supposed to go, connect up and become functional…is a bigger problem in the nervous system than anywhere else"

Mark Mattson, *NINDS, Bethesda*





Genetic engineering: fear and worry

"Went in for a simple blood test and got cloned by mistake."