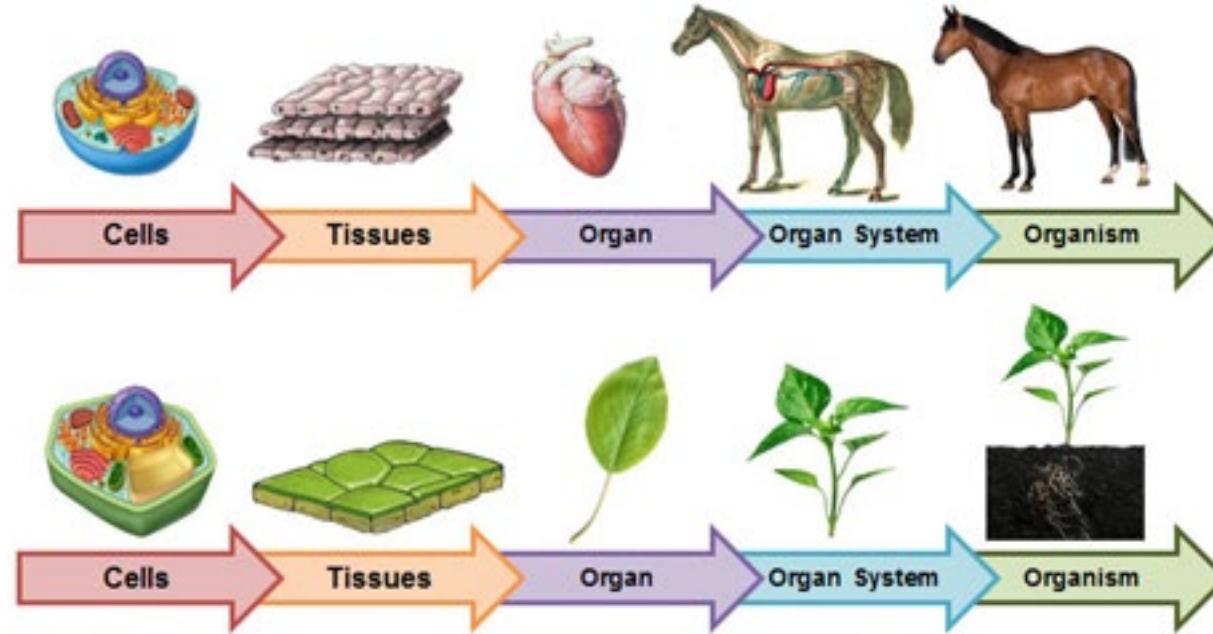


LSM1301



Biotechnology

Maxine Mowe

Office at S2-04

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Tel: 65161614

Lecture Topics and Assignments

- Introduction (1 Lecture Session)
- Dr. NP Lectures/labs/Museum (5 Lects, 2 Labs, 1 Museum, 3 Tutorials)
 - Total Assignments (25%)
- Chemistry of Life (1 Lect session)
- Cell Structure and Function (1 Lect)
- Energy and Life (1 Lect)
- DNA and Gene Expression (2)
- Biotechnology (1 Lect)
- Summary & Tutorial (1 Session: 10am, 7th April)
 - Total Assignments 25% = 4 lab assignments (25%)



Today

Learning Plan (Biotechnology)

Topic	Learning outcomes for the WK	Activities for online session	Activities	Assignments/ Assessments
Biotech-nology	<ul style="list-style-type: none"> • Explain the principle of PCR • Apply PCR for gene cloning and forensic sciences • Analyze and interpret the image data of DNA agarose gel electrophoresis • Explain the general procedure for gene cloning • Explain how DNA can be cleaved and re-joint to form recombinant DNAs • Use STRs as fingerprinting for identities • Apply the knowledge of DNA and biotechnologies for detection of genetic disorders 	<ul style="list-style-type: none"> • Read lecture notes to gain an overall picture about this topic • Watch video: PCR https://www.youtube.com/watch?v=a5jmdh9AnS4) • Watch video: Restriction Enzymes Pt 1 https://www.youtube.com/watch?v=lPdQwdGgyfQ&nohtml5=False (10 min) • Biotechnology - Cloning & Genetic Engineering http://www.youtube.com/watch?v=XTFNbeNC4pA (14 min) DNA Fingerprinting http://www.youtube.com/watch?v=DbR9xMXuK7c (7 min) 	<ul style="list-style-type: none"> • Demonstrate how to use microscopes; how to perform PCR, set program for PCR; how to prepare agarose gel and to conduct electrophoresis • Engage students with microinjection, showing how gene can be modified • Discuss about gene modified organisms: technologies and its socio-economic impacts • Discuss about genetic screening (for example prenatal examination) and gene therapy and ethics 	<ul style="list-style-type: none"> • Enhance understanding with Track-learning MCQs • Watch webcast lecture if needed

Intended Learning Outcomes

- At the end of this class, the student should be able
 - To describe the processes involved in the generation of genetically modified organisms, and to relate the differences between the processes for bacteria, plants, and animals to concepts acquired in the topics of ‘Cell Structure and Function’, ‘DNA and Heredity’ and ‘Gene Expression’
 - To describe the methodologies used in DNA profiling, and to relate the principles of the methodologies with concepts acquired in the topics of ‘Chemistry of Life’, ‘Cell Structure and Function’, ‘DNA and Heredity’ and ‘Gene Expression’
 - To apply the concepts of genetically modified organisms and DNA profiling to real-life examples, such as transgenic bacteria, plants and animals, genetic screening and gene therapy
 - To reflect on the environmental, safety and ethical issues from the use of biotechnology

Outline

- Genetically Modified Organisms
 - Transgenic bacteria
 - Transgenic plants
 - Transgenic animals
- DNA Profiling
 - Polymerase chain reaction
 - Gel electrophoresis
- DNA probes
- DNA chips
- Genetic screening
- Gene Therapy
- Issues

Outline

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Biotechnology

In its broadest sense, biotechnology is any use or alteration of organisms, cells or biological molecules to achieve specific practical goals.”

- Ancient biotechnology:
 - Fermentation (beer, bread, wine), domestication, selective breeding
- Modern biotechnology ≈ Genetic engineering
 - Manipulates genetic information in an organism



a model of a bakery before
century around 1782

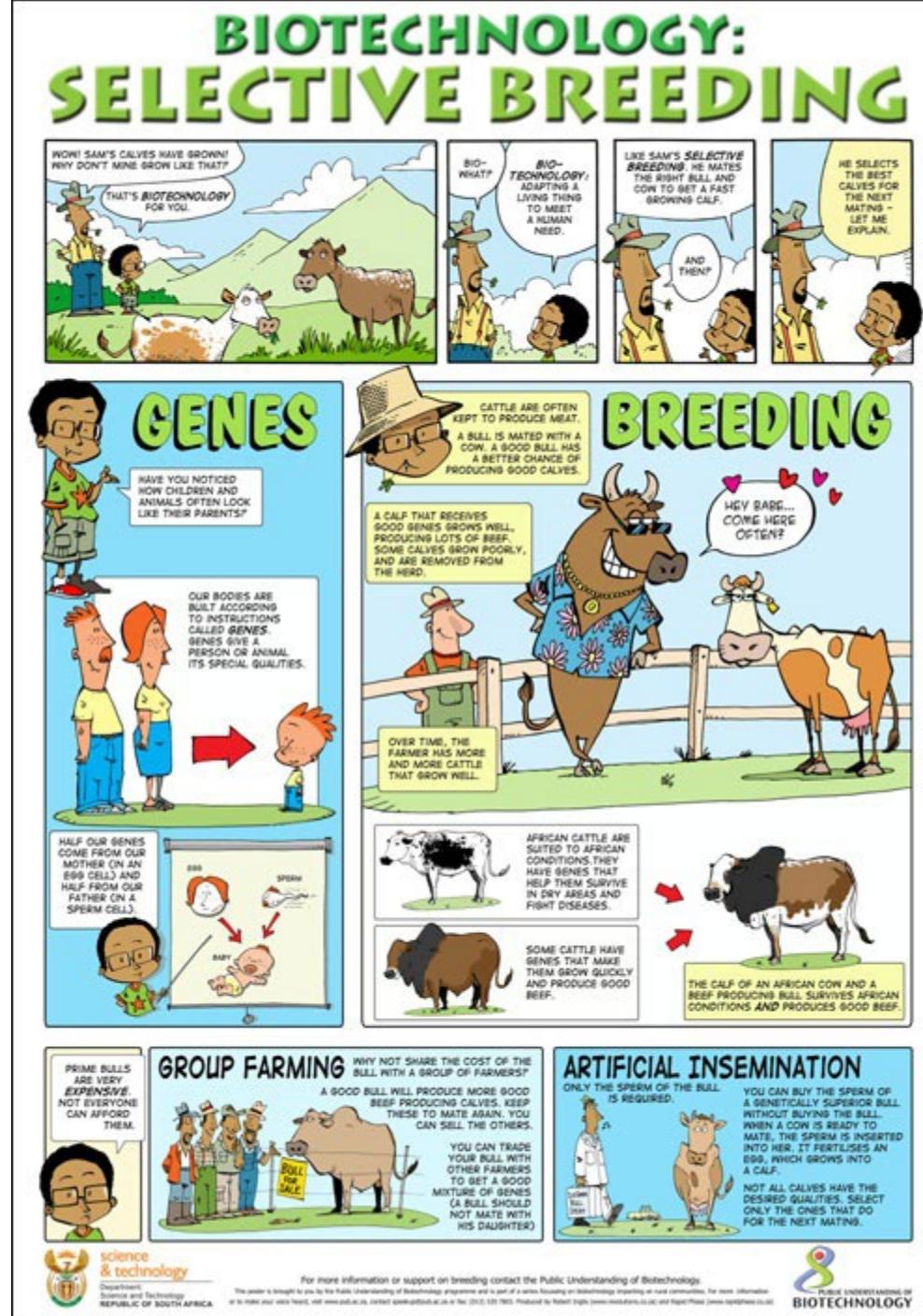


MICE EXPRESSING GFP

Courtesy of Advanced Cell Technology

Ancient biotechnology

- Use of biology and living organisms for practical purposes
- Traditional examples
 - Beer-brewing
 - Wine-making
 - Animal breeding
 - Plant breeding



Genetically Modified Organisms

- Organisms with deliberately modified genes of another individual of the same species
- Also include organisms with genes from another species
 - Transgenic organisms
- Made possible by DNA cloning
 - Generation of identical copies of specific DNA fragments in large quantities

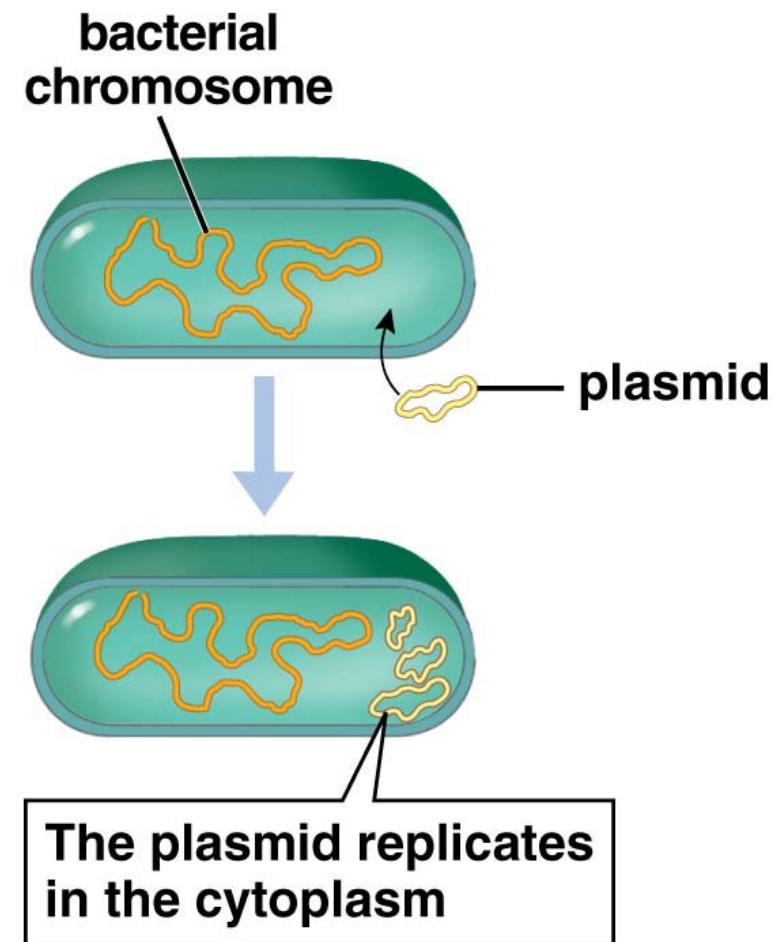


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Genetically Modified Organisms

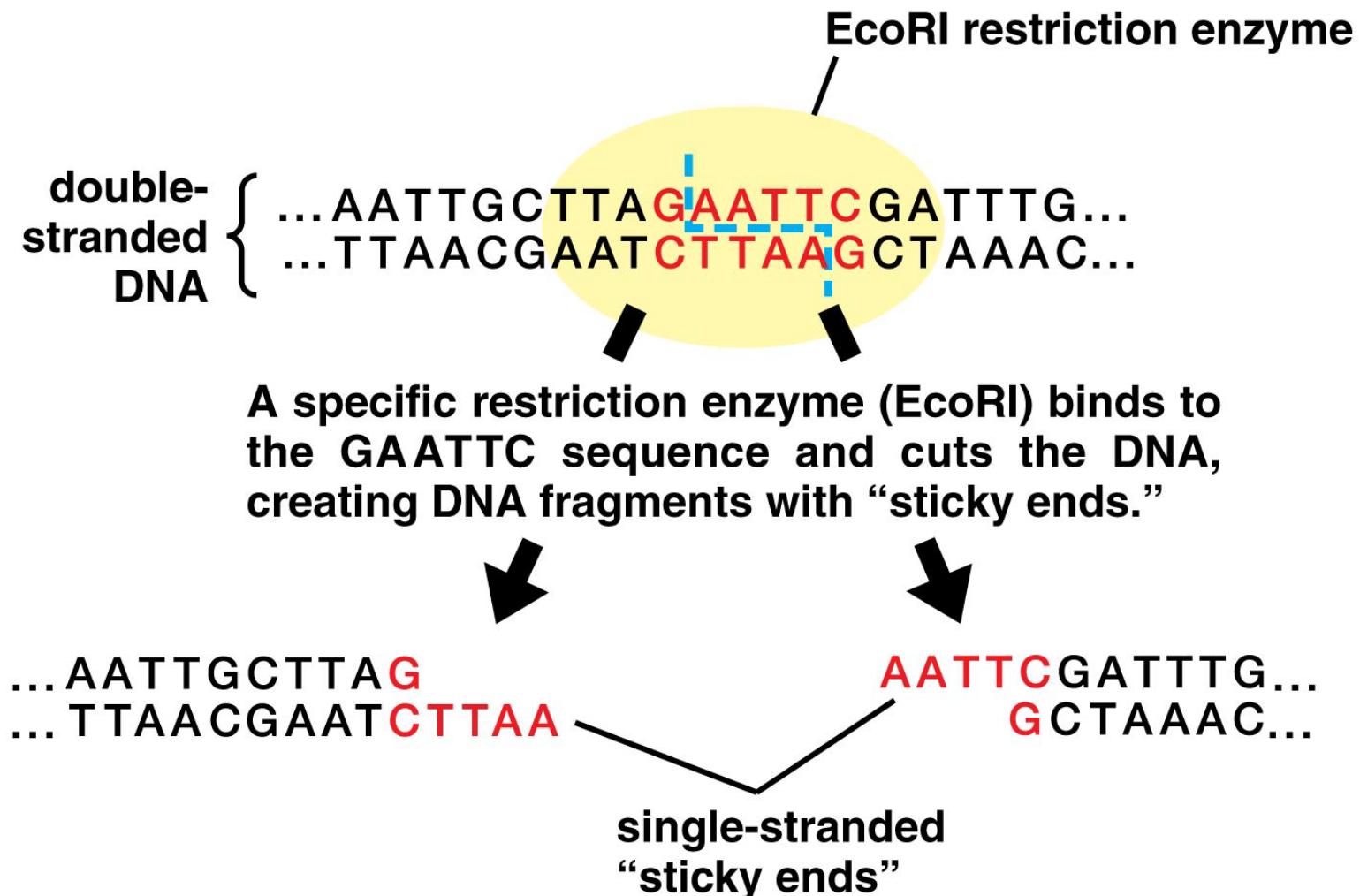
- DNA cloning requires cloning vector
 - Small piece of DNA to carry source DNA into host cell
 - Replicates to produce many copies of source DNA
 - Common vectors – bacterial plasmids



(b) Transformation with a plasmid

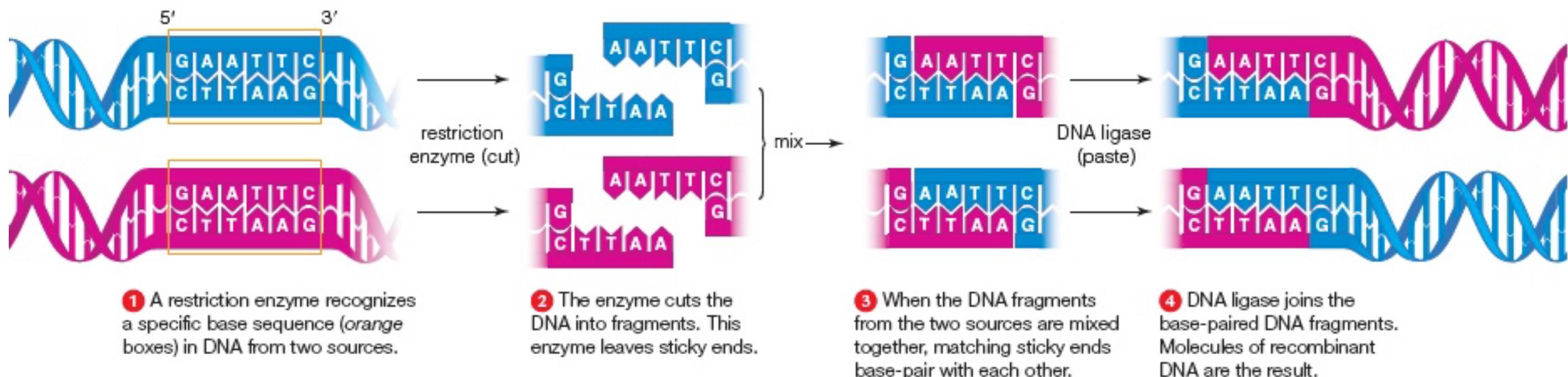
Genetically Modified Organisms

- Restriction enzymes
 - Bacterial enzymes that cleave DNA at specific nucleotide sequences
 - Hundreds of different restriction enzymes, cleaving at different sequences



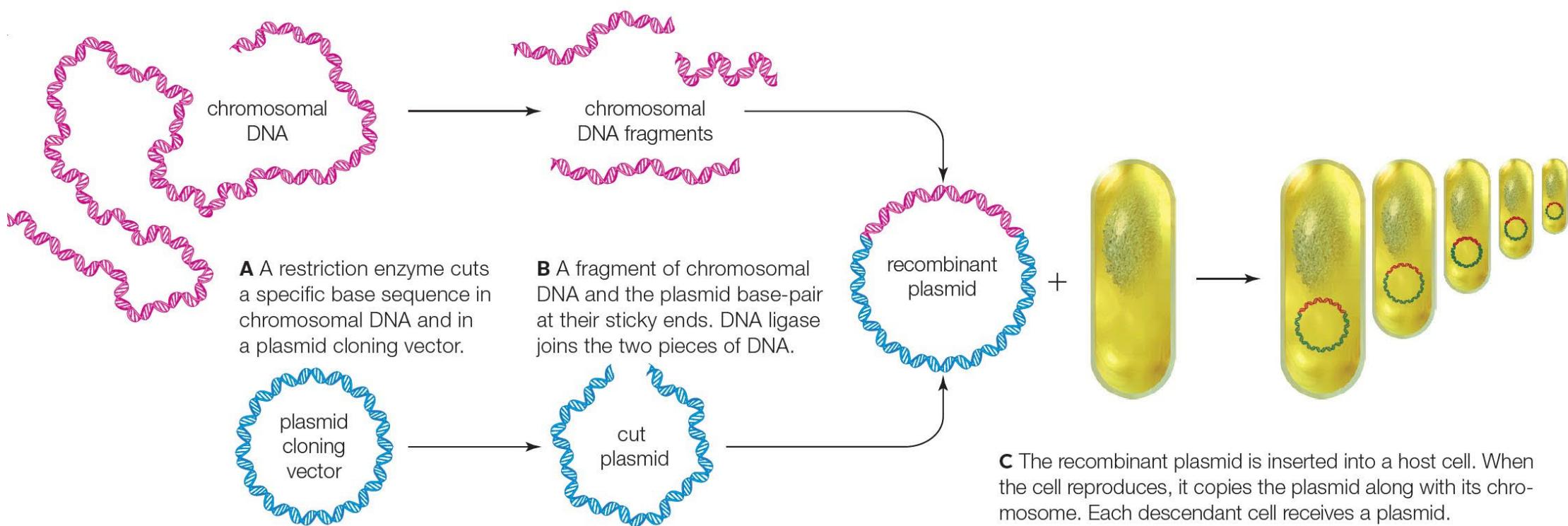
Genetically Modified Organisms

- Same restriction enzyme used to cleave both plasmid and source DNA
 - Ends of cleaved plasmid and source DNA have complementary nucleotides
 - Complementary nucleotides base-pair together



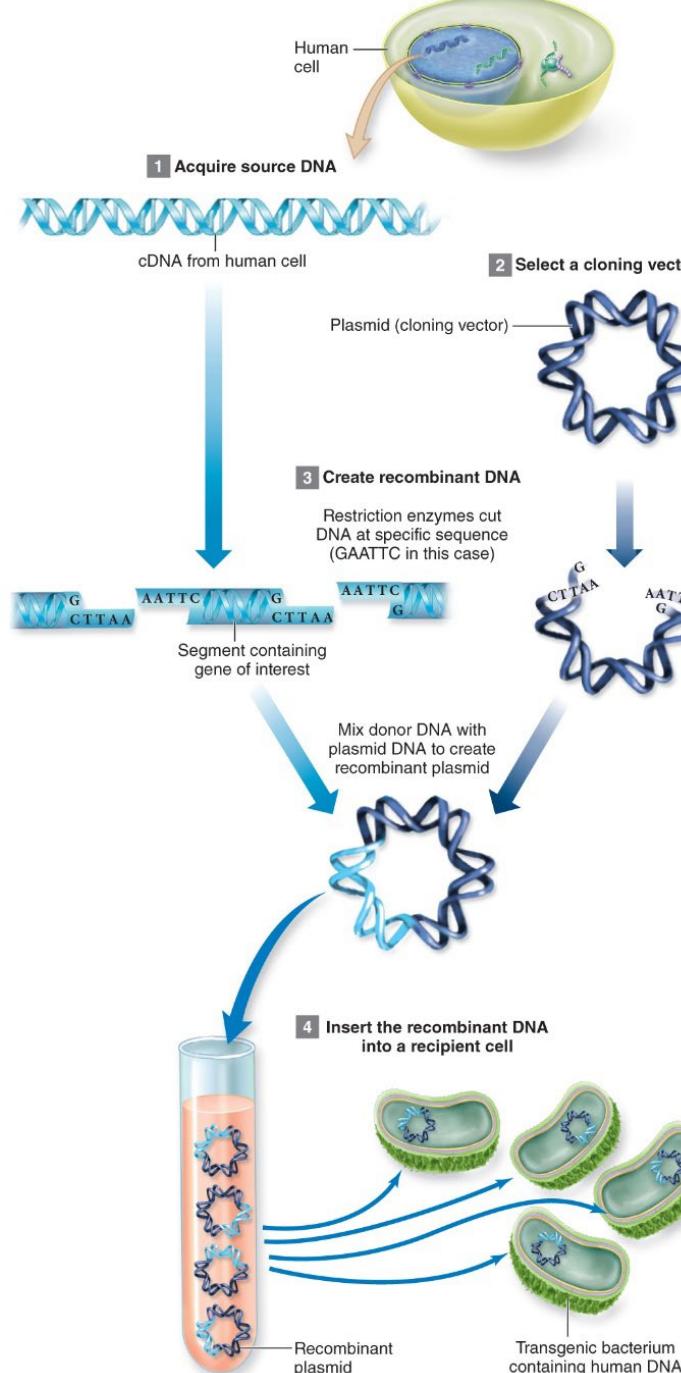
Genetically Modified Organisms

- DNA ligase
 - Seals the base-paired plasmid and source DNA
 - Recombinant DNA (DNA from two or more different organisms)



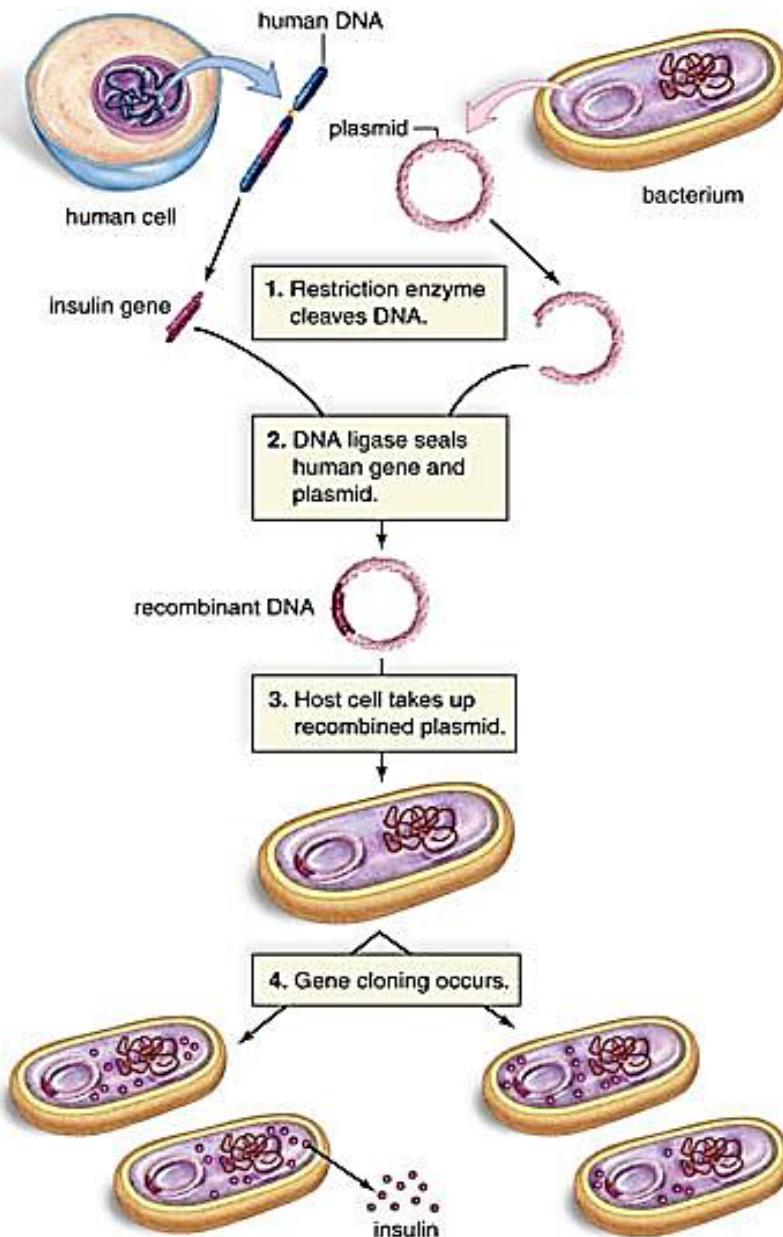
Genetically Modified Organisms

- Recombinant DNA
 - Inserted into host cell
 - Host cells are treated to enable take up of recombinant plasmids
 - Both host bacterial cells and recombinant plasmids replicate, producing many copies of source DNA



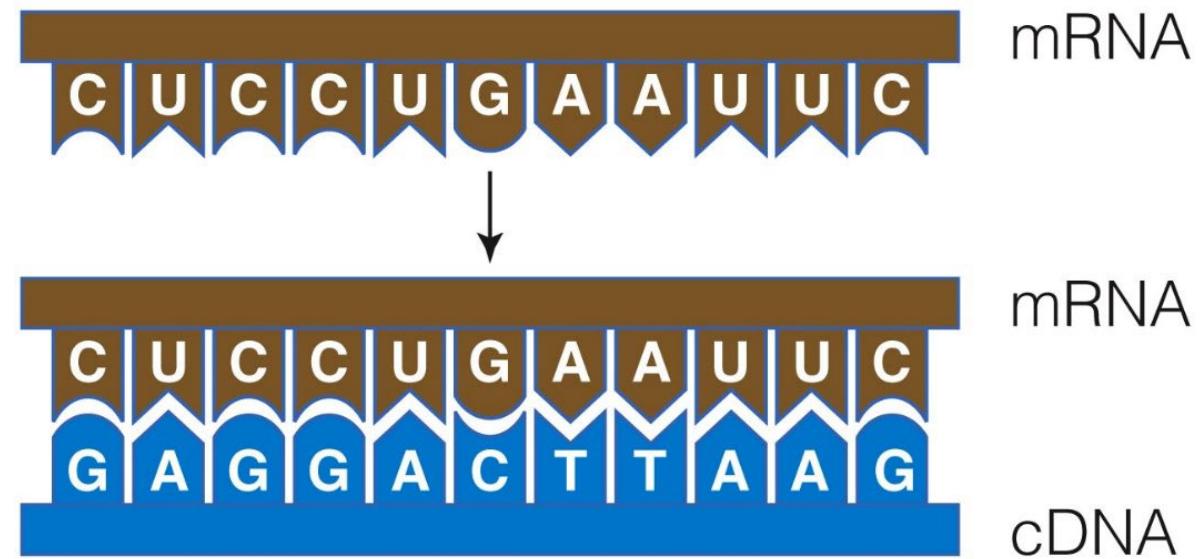
Transgenic Bacteria

- Gene of interest inserted into bacteria
 - Transgenic bacteria grown in large bioreactors and gene product harvested
- Eukaryotic genes have introns
 - Bacterial cells cannot process pre-mRNA
- Use mature mRNA for cloning
 - Reverse transcriptase transcribes mRNA to DNA
 - Hybrid RNA-DNA molecule formed



Transgenic Bacteria

- DNA polymerase forms double stranded DNA molecule
 - Can be used for cloning in bacterial cells
- Resulting DNA
 - Copy of mature mRNA (without introns)
 - Known as complementary DNA (cDNA)



Transgenic Bacteria

- Uses of transgenic bacteria
 - To produce medically important proteins – human insulin, hepatitis B, vaccine, human growth hormone, blood-clotting factors, etc.
 - To produce fluorescent protein from jellyfish – to study gene expression
 - To produce enzymes – to improve taste and clarity of beer and fruit juice, to slow bread staling, to modify fats
 - To degrade substances – oil-eating bacteria, to clean-up toxic waste



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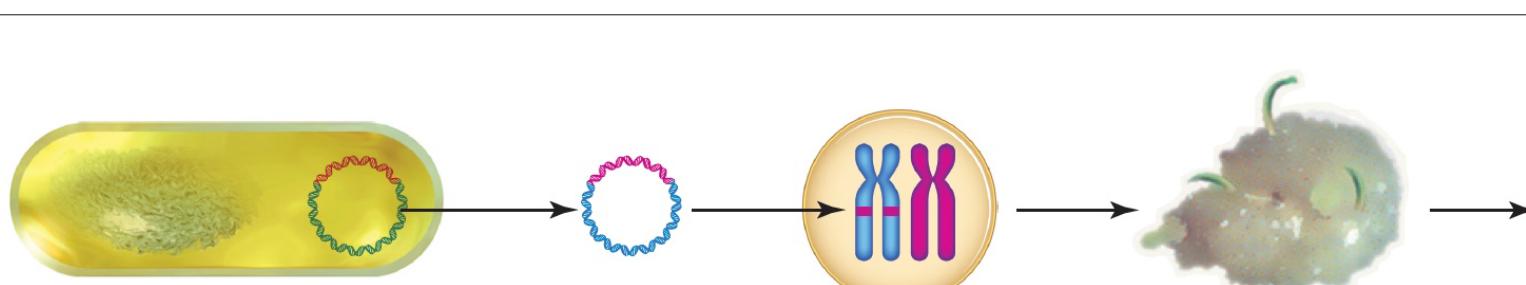
© U.S. Coast Guard photo by Petty Officer 3rd Class Patrick Kelley

Transgenic Plants

- Foreign or modified genes can be introduced into
 - Plant embryos
 - Protoplasts – plant cells with cell walls removed
- Introduction of genes into plant cells
 - ‘Gene gun’ – shooting with DNA-coated gold or tungsten microscopic pellets
 - Ti (tumour-inducing) plasmid of plant-infecting bacterium *Agrobacterium tumefaciens*
- When *A. tumefaciens* infects plant cells
 - Tumour-causing genes of Ti plasmid inserted into plant chromosome

Transgenic Plants

- Researchers replace tumour-causing genes with foreign or modified genes
- When *A. tumefaciens* with modified Ti plasmid infects plant cells
 - Foreign or modified genes transferred into plant chromosome



A A Ti plasmid carrying a foreign gene is inserted into an *Agrobacterium tumefaciens* bacterium.

B The bacterium infects a plant cell and transfers the Ti plasmid into it. The plasmid DNA becomes integrated into one of the cell's chromosomes.

C The plant cell divides, and its descendants form an embryo. Several embryos are sprouting from this mass of cells.



D Each embryo develops into a transgenic plant that expresses the foreign gene. The glowing tobacco plant is expressing a gene from fireflies.

Transgenic Plants

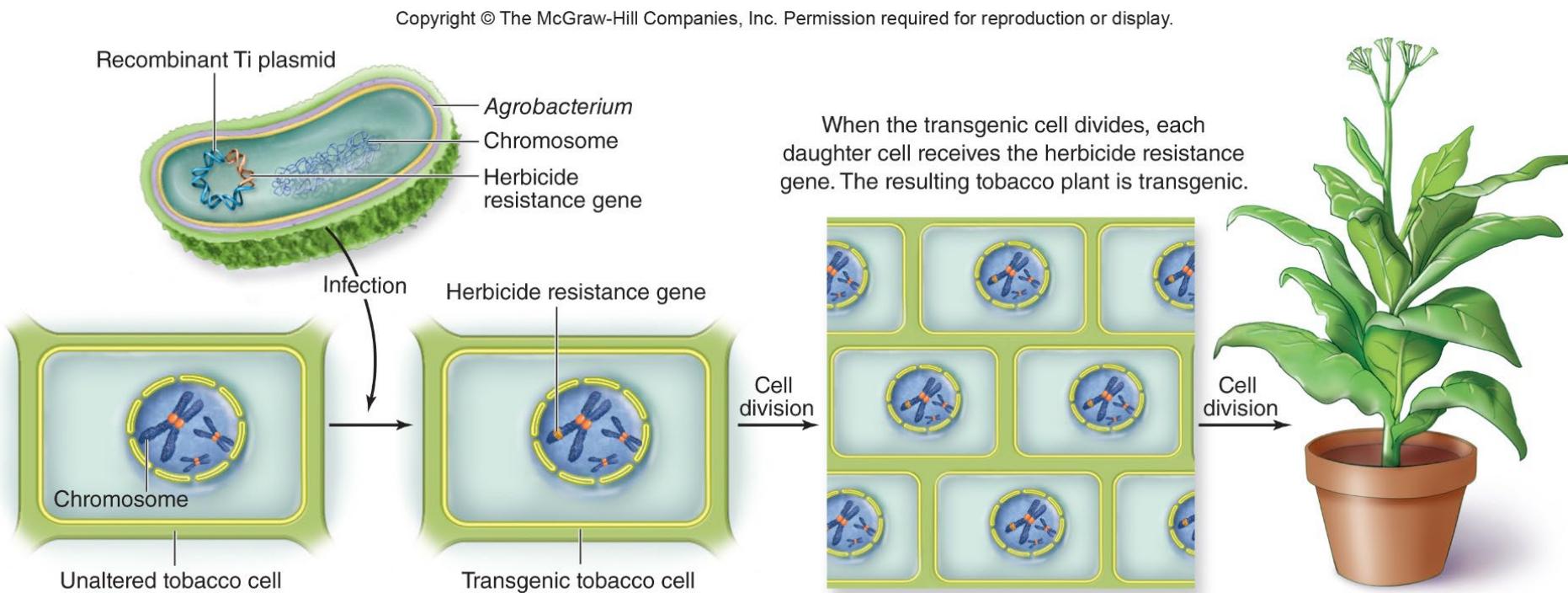
- Insect resistant crops
 - Possess Bt gene from bacterium *Bacillus thuringiensis*
 - Gene encodes protein that damages digestive tracts of insects
 - Bt genes have been inserted in cotton, corn and potato



Bt gene (from *Bacillus thuringiensis* bacterium) can be inserted into plants to produce insect-killing protein in crops

Transgenic Plants

- Herbicide resistant crops
 - Resistant to weed-killing chemicals
 - Herbicide resistant soybeans and potato



Transgenic Plants

- Plants with medically useful gene
 - Producing harmless hepatitis B virus that stimulate immune response when eaten
 - Producing human antibodies that confer immunity to microbial infection when eaten
 - Producing other human proteins, e.g. hormones, clotting factors, etc.
- More nutritious food plants
 - Rice plants that produce β -carotene (precursor of vitamin A)



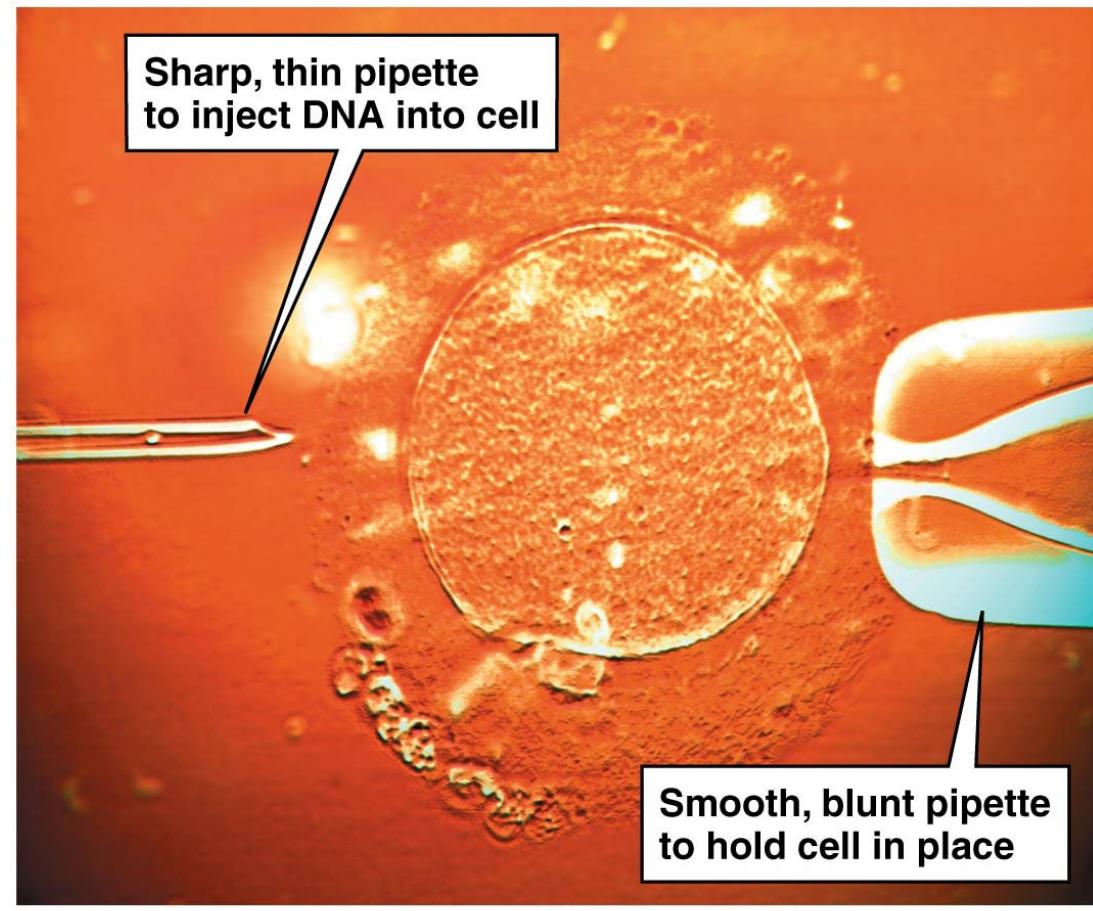
Table 13-1 Genetically Engineered Crops with USDA Approval

Genetically Engineered Trait	Potential Advantage	Examples of Bioengineered Crops with USDA Approval
Resistance to herbicide	Application of herbicide kills weeds but not crop plants, producing higher crop yields	Beet, canola, corn, cotton, flax, potato, rice, soybean, tomato
Resistance to pests	Crop plants suffer less damage from insects, producing higher crop yields	Corn, cotton, potato, rice, soybean
Resistance to disease	Plants are less prone to infection by viruses, bacteria, or fungi, producing higher crop yields	Papaya, potato, squash
Sterile	Transgenic plants cannot cross with wild varieties, making them safer for the environment and more economically productive for the seed companies that produce them	Chicory, corn
Altered oil content	Oils can be made healthier for human consumption or can be made similar to more expensive oils (such as palm or coconut)	Canola, soybean
Altered ripening	Fruits can be more easily shipped with less damage, producing higher returns for the farmer	Tomato

In 2008, about 80% of the corn, 86% of the cotton, and 92% of the soybeans grown in the U.S. were transgenic (contained the genes from other species)

Transgenic Animals

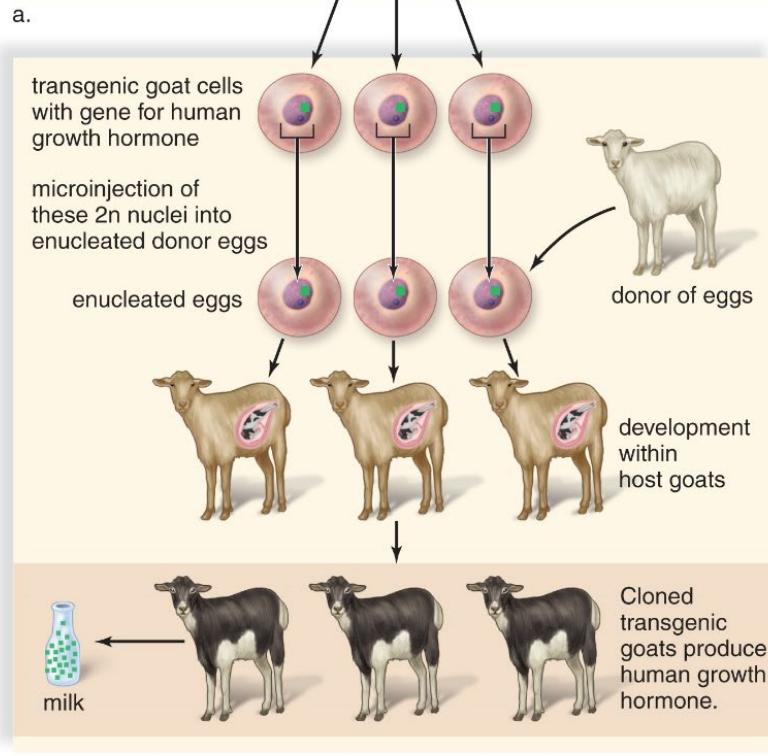
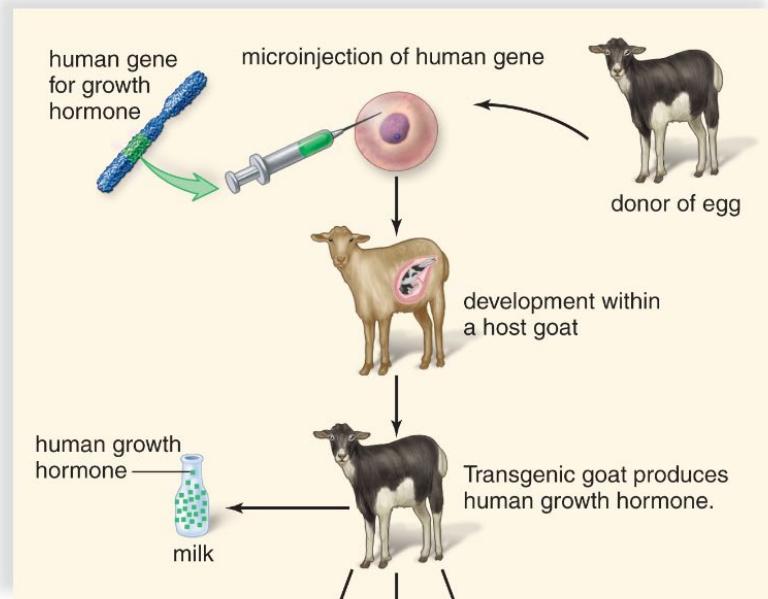
- New DNA inserted into fertilised egg
 - Infection by disabled viruses carrying foreign or modified genes
 - Direct microinjection of foreign or modified genes
 - Delivery of foreign or modified genes via temporary punctures on plasma membranes by silicon-carbide needles in a rotating mixer
- Inefficient process
 - Success rate of about 1%
- Requires normal expression
 - Must not disrupt other essential genes
 - Must be expressed at right cells and right time



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Transgenic Animals

- Animals that produce proteins for industrial applications
 - Goats that produce spider silk (that produces a meshwork stronger than steel or Kevlar) in their milk
- Animals that produce medically important proteins
 - Sheep and goats that produce proteins for treating cystic fibrosis and blood clotting disorders in their milk
 - Rabbits that produce human interleukin-2 (for treatment of some cancers) in their milk
 - Goats that produce lysozyme (that protects developing nations' infants and children from acute diarrhoeal disease) in their milk



Transgenic Animals

- Animals with altered qualities
 - Larger fishes, cows, pigs, rabbits and sheep that possess gene for bovine growth hormone
 - Pigs that produce omega-3 fatty acids
 - Chickens that cannot spread H5N1 influenza virus
- Animals for scientific research
 - Mice that serve as models of human diseases
 - Mice used to reveal functions of human genes



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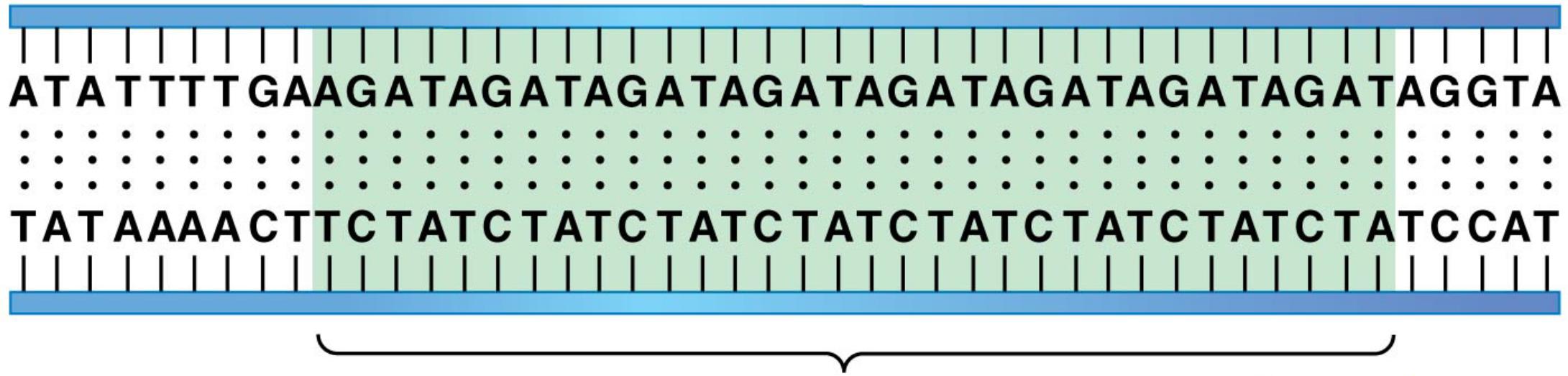
Outline

- Genetically Modified Organisms
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DNA Profiling

- One DNA profiling method – analysis of short tandem repeats (STRs)
 - Short segments of DNA of 2 to 5 nucleotides, repeated several times (5 to 50 times), in tandem (alongside one another in a row)
 - Sequences that do not code for proteins, found on many sites in genome
 - Vary greatly among individuals, like genetic fingerprints
 - Each person carries a unique combination of STRs
 - Chance of two non-identical twins having identical STRs in 3 regions of DNA is 1 in 10^{18}
- Many different applications – paternity suits, rape cases, corpse identification, population studies, etc.

DNA Profiling

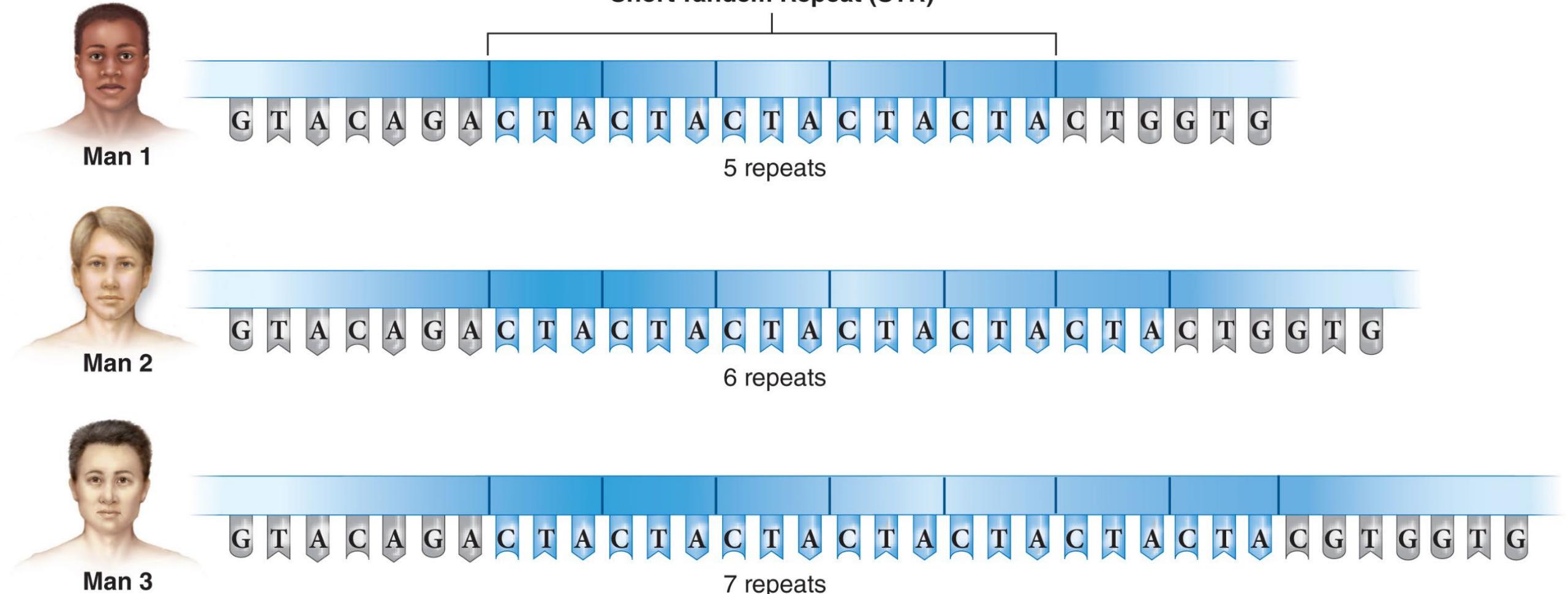


**Eight side-by-side (tandem) repeats
of the same four-nucleotide sequence**

DNA Profiling

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Short Tandem Repeat (STR)

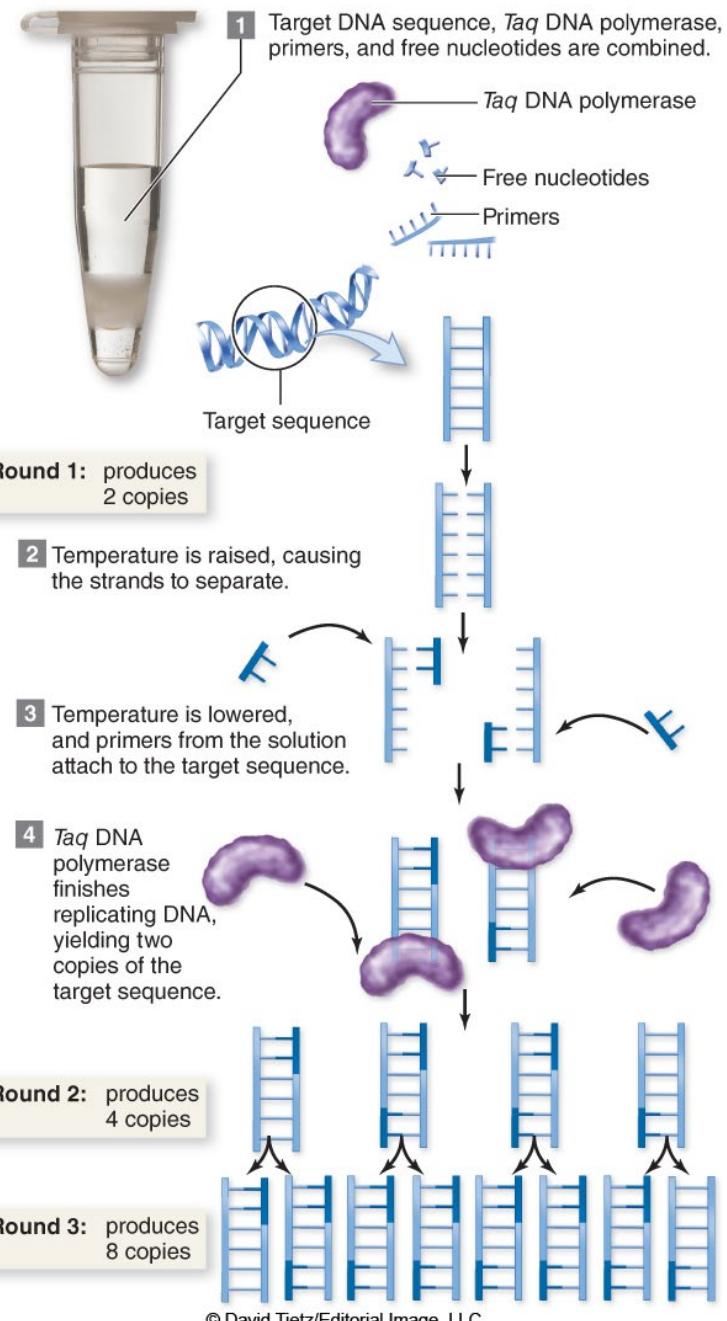


DNA Profiling

- Polymerase chain reaction (PCR) used to amplify sample DNA (e.g. crime scene) from regions of with STRs
- Cleave amplified DNA segments with restriction enzymes
 - Unique collection of fragments of different lengths produced
- Gel electrophoresis used to separate fragments
 - Unique banding pattern produced
 - Called DNA fingerprint
- DNA probes used to identify STRs in gel

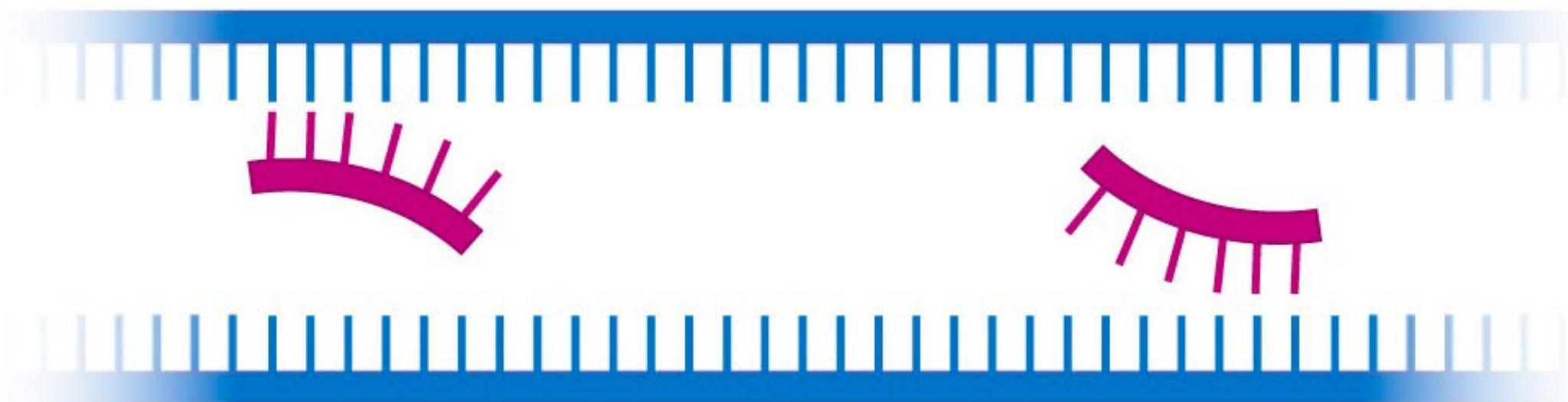
Polymerase Chain Reaction

- A cycled reaction that uses a heat-tolerant form of DNA polymerase (Taq polymerase)
 - Creates billions of copies of a DNA fragment in a test tube
- Requires knowledge of nucleotide sequence
 - For design of a pair of short pieces of DNA (called **primers**) complementary to targeted sequence of DNA for amplification
- Involves 2 major steps
 - Identifying DNA segment to be amplified
 - Amplifying targeted DNA by running cycled reactions



Polymerase Chain Reaction

- DNA with target fragments mixed with
 - Heat-tolerant DNA polymerase (*Taq* polymerase)
 - Synthesised DNA primers
 - Free DNA nucleotides

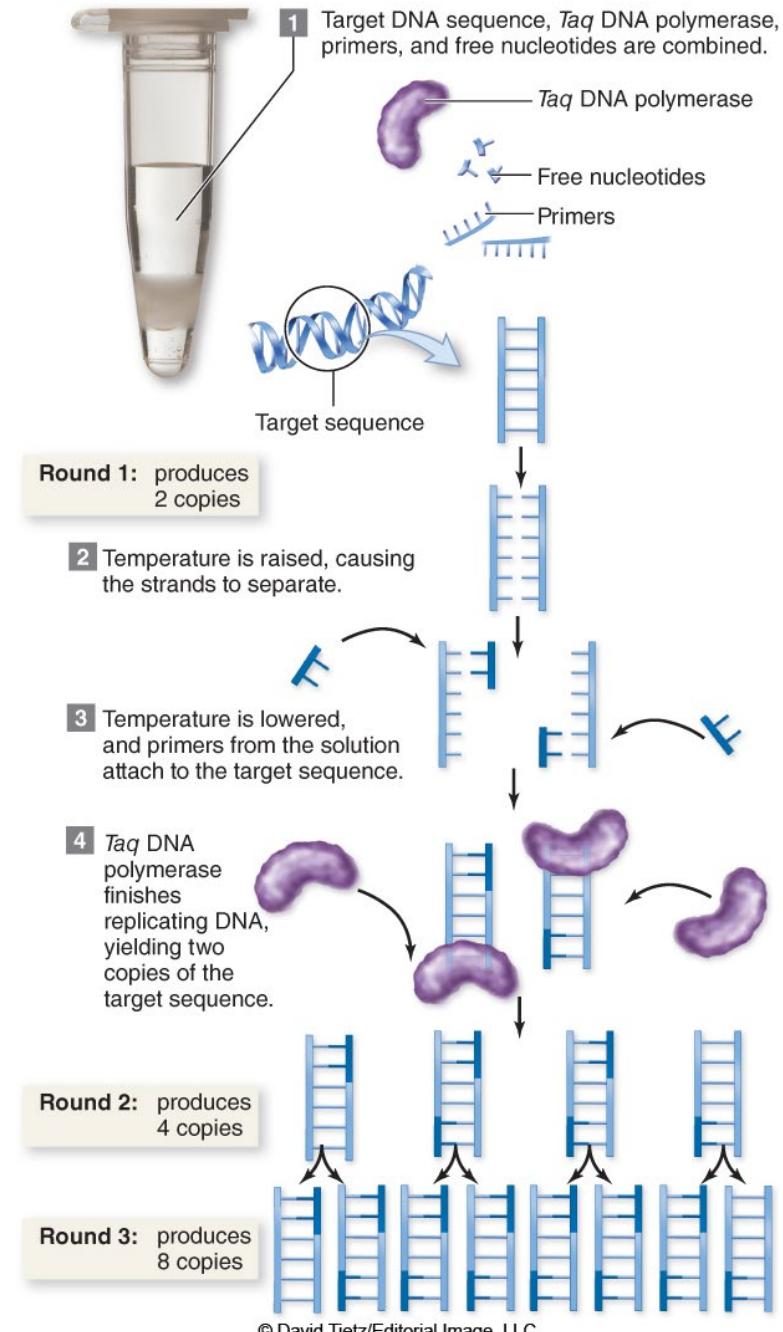


Polymerase Chain Reaction

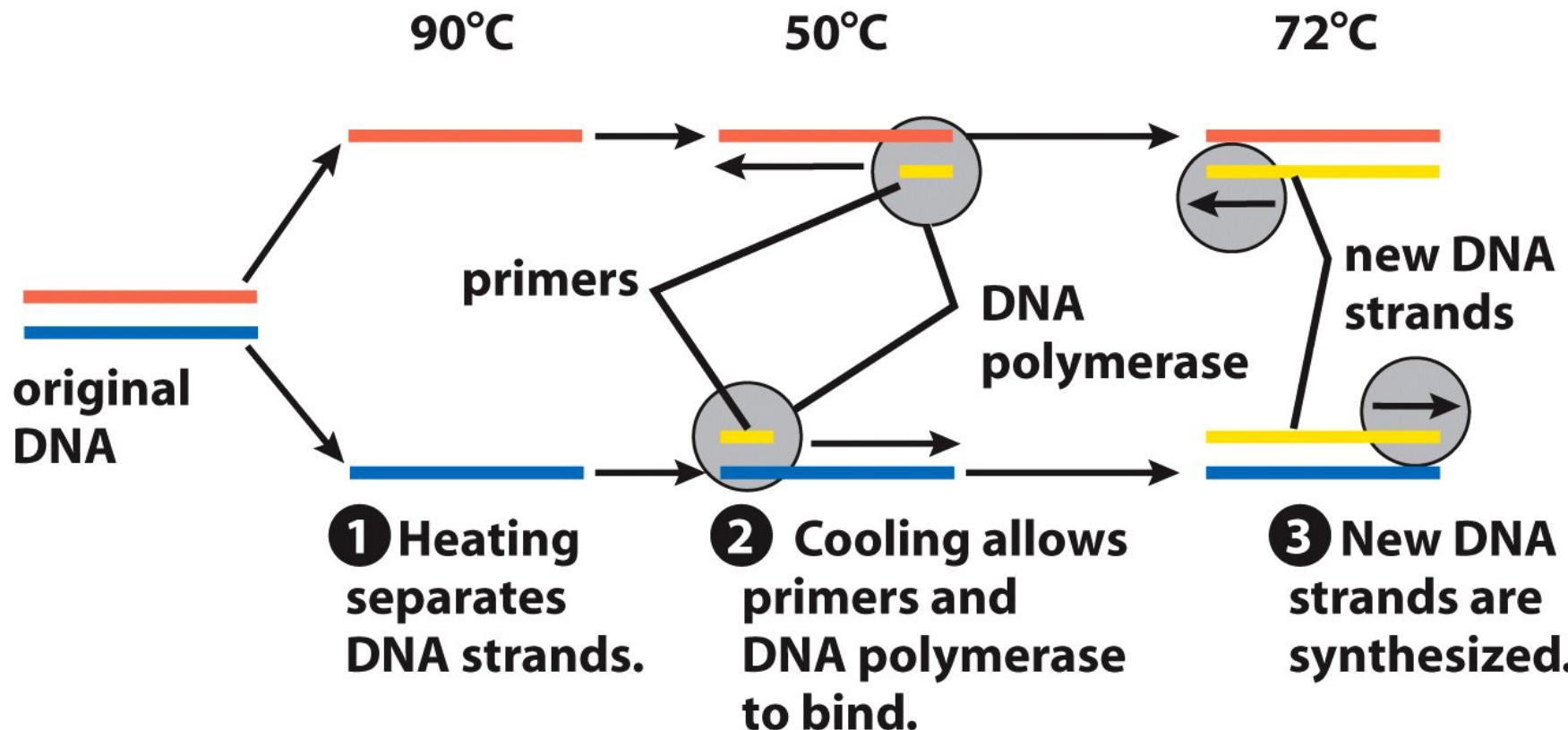
Denaturation: Targeted DNA sequence to be amplified heated to 90-95°C to separate DNA to single strands

Annealing: Temperature lowered to 50°C to allow primers to bind to single DNA strands

Extension: Temperature raised to 70-72°C for DNA polymerase to use free nucleotides to synthesize complementary strands



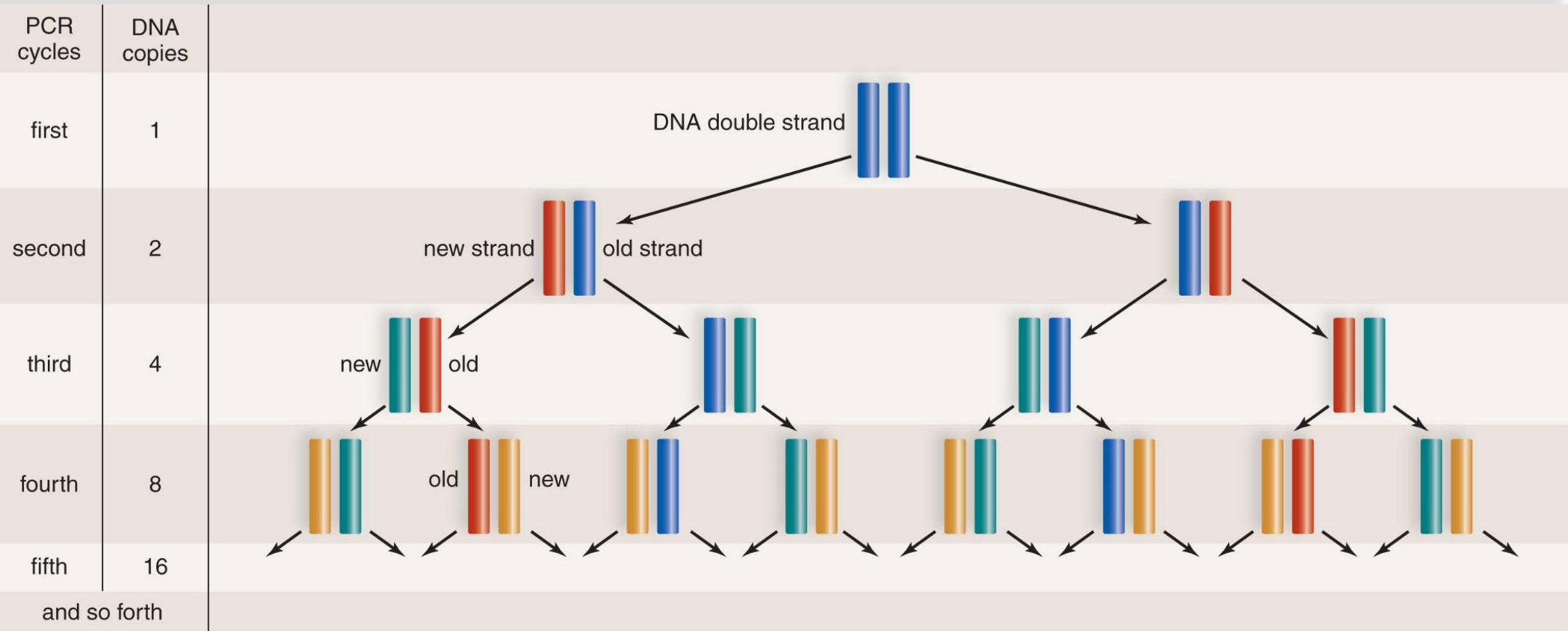
One PCR cycle



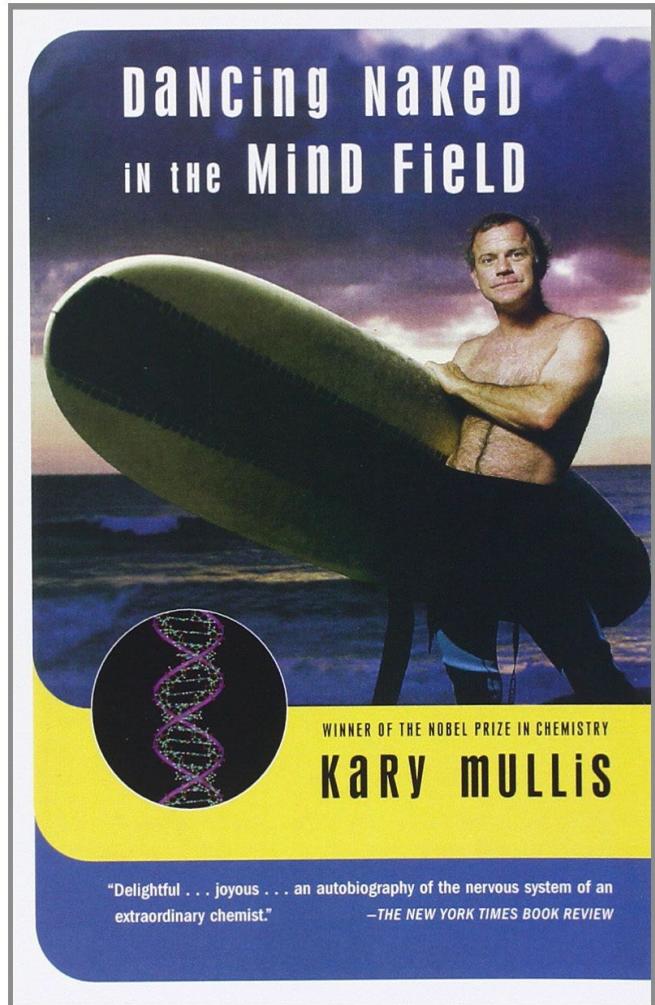
PCR begins with a mixture containing a **DNA template**, a pair of short ssDNA oligonucleotide **primers**, a pool of the four **dNTPs**, and a heat-resistant DNA polymerase, **Taq Enzyme**

Polymerase Chain Reaction

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Polymerase Chain Reaction

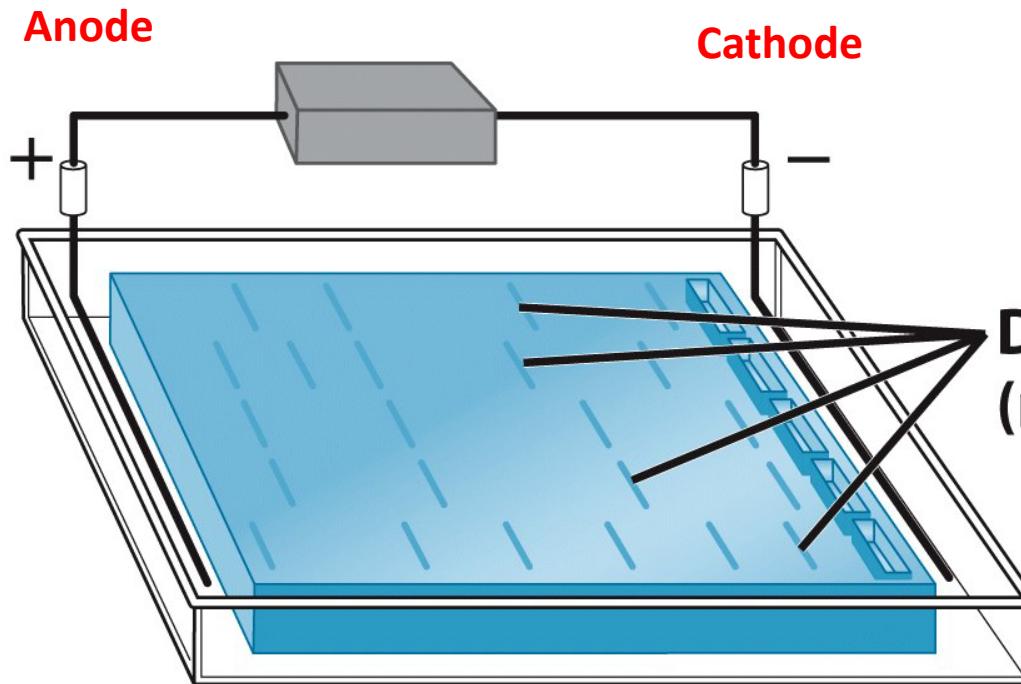


- Developed by Kary B. Mullis
- Nobel Prize in Chemistry 1993
 - ‘for his invention of the polymerase chain reaction (PCR) method’
- Personal website
 - <http://www.karymullis.com/>
- Book
 - Dancing Naked in the Mind Field
 - Published, 1998
 - NUS Science Library – Q173 Mul

Gel Electrophoresis

- After PCR and cleaving by restriction enzymes, DNA fragments of varying lengths are obtained
- DNA fragments can be separated on the basis of charge and length (or size) by gel electrophoresis
- Steps of gel electrophoresis
 - DNA mixtures placed into wells at one end of gel
 - Electric current applied to gel – negatively-charged DNA moves toward positive end of gel, with smaller fragments faster
- Separated bands of DNA made visible by stains or DNA probes

Gel casting



**Electrical current moves DNA segments through the gel.
Smaller pieces of DNA move farther toward the positive electrode.**



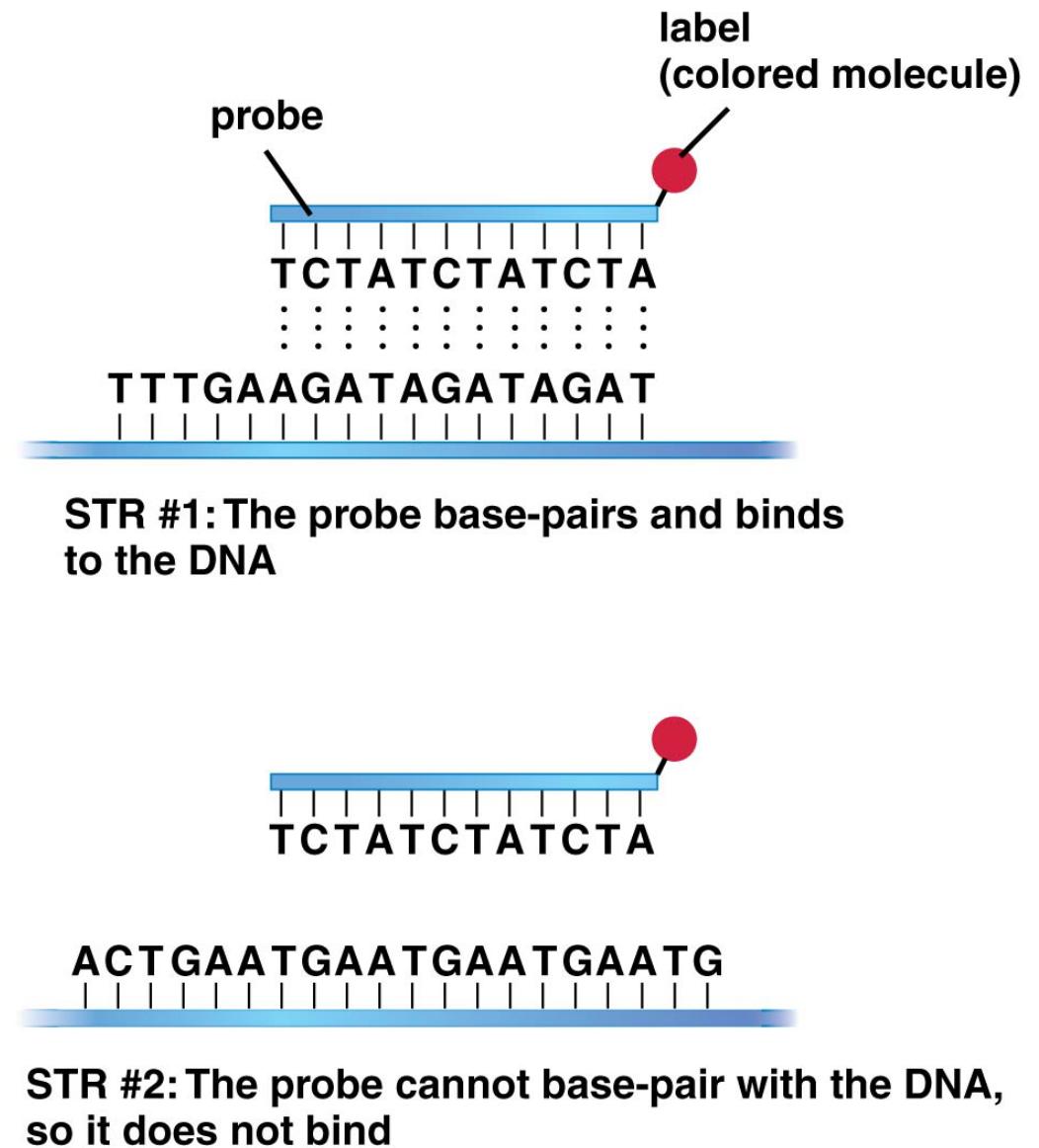
molten agarose

(Agarose) Jelly Food



DNA Probes

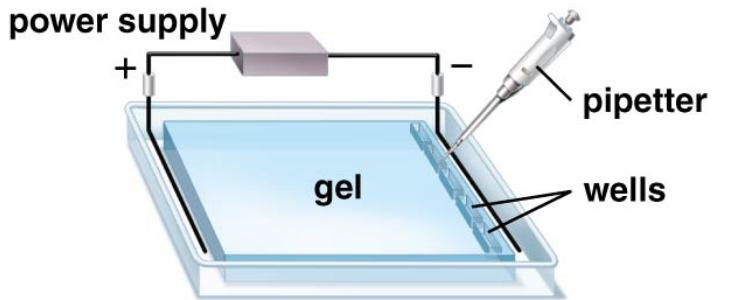
- Short single-stranded DNA fragments used to label specific nucleotide sequences
 - Nucleotide sequence of DNA probe complementary nucleotide sequence of DNA fragments to be labelled
 - DNA probe labelled with radioactivity or coloured fluorescent dye to mark location of DNA fragments that are base-paired to probe



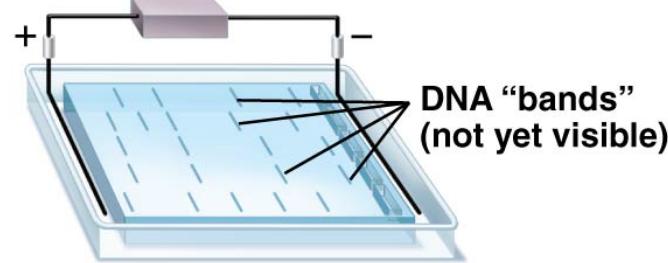
DNA Probes

- For DNA profiling, at the end of gel electrophoresis, DNA fragments separated into single strands
 - Current applied in different direction transfers separated DNA bands down onto nylon filter
- Nylon filter with DNA bands on it then bathed in solution of labelled DNA probes
 - DNA probes complementary to specific DNA nucleotide sequences of interest (e.g. specific STR)
- Targeted DNA bands base-pair with probe
 - Location of target DNA bands on nylon filter revealed

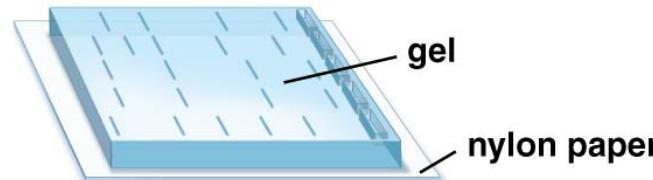
1 DNA samples are pipetted into wells (shallow slots) in the gel. Electrical current is sent through the gel (negative at the end with the wells and positive at the opposite end).



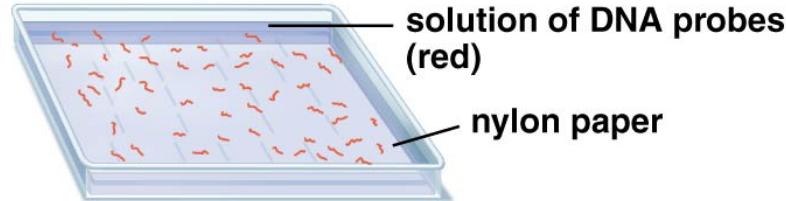
2 Electrical current moves the DNA segments through the gel. Smaller pieces of DNA move farther toward the positive electrode.



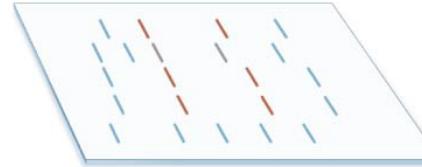
3 The gel is placed on special nylon "paper." Electrical current drives the DNA out of the gel onto the nylon.

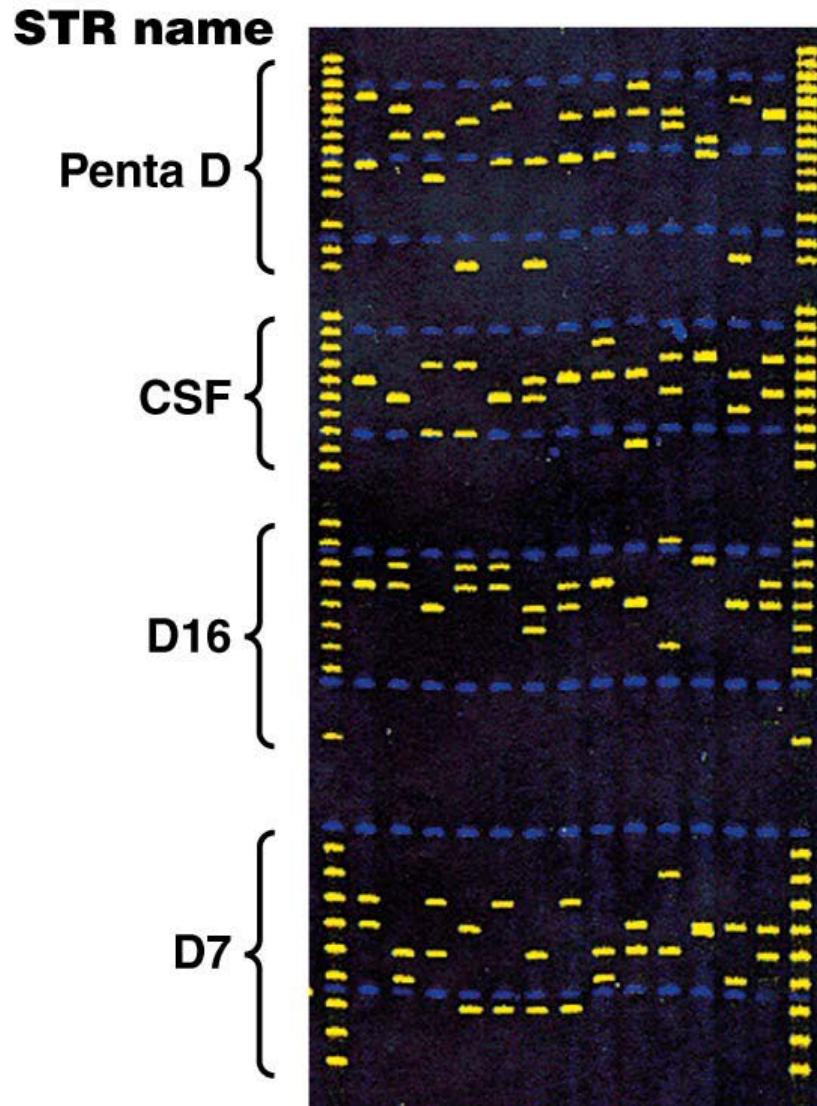


4 The nylon paper with the DNA bound to it is bathed in a solution of labeled DNA probes (red) that are complementary to specific DNA segments in the original DNA sample.



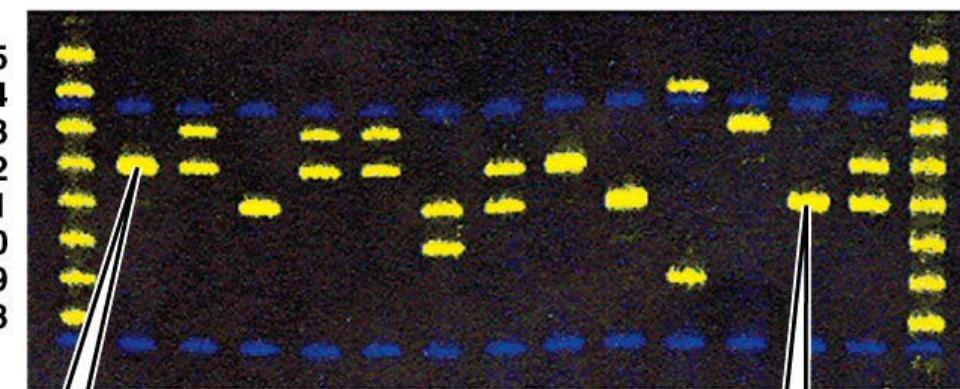
5 Complementary DNA segments are labeled by the probes (red bands).





Number of repeats

D16: An STR on chromosome 16



DNA samples from
13 different people

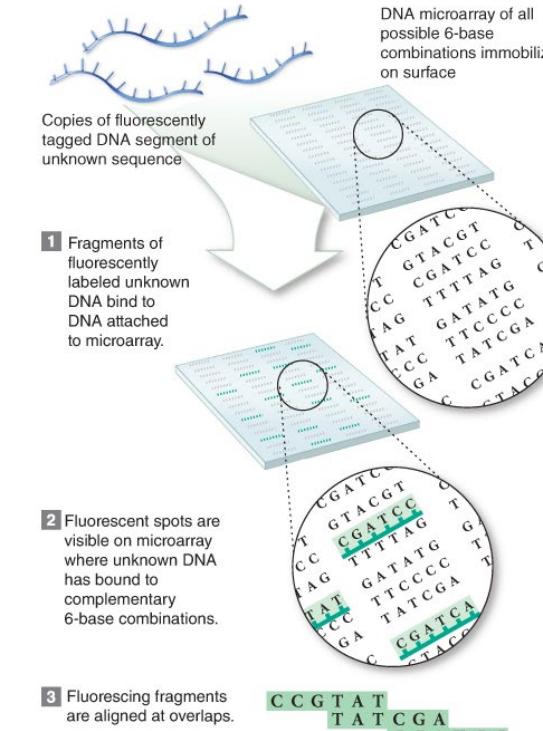
D16 in this person
contains 12 repeats

D16 in this person
contains 11 repeats

- Unrelated people almost never have identical DNA profiles
- The positions of the bands on the gel are determined by the numbers of repeats in each STR
- Using the standard array of STRs, technicians determine the criminal's DNA profile, which is coded by the number of repeats of each STR found in the criminal's DNA

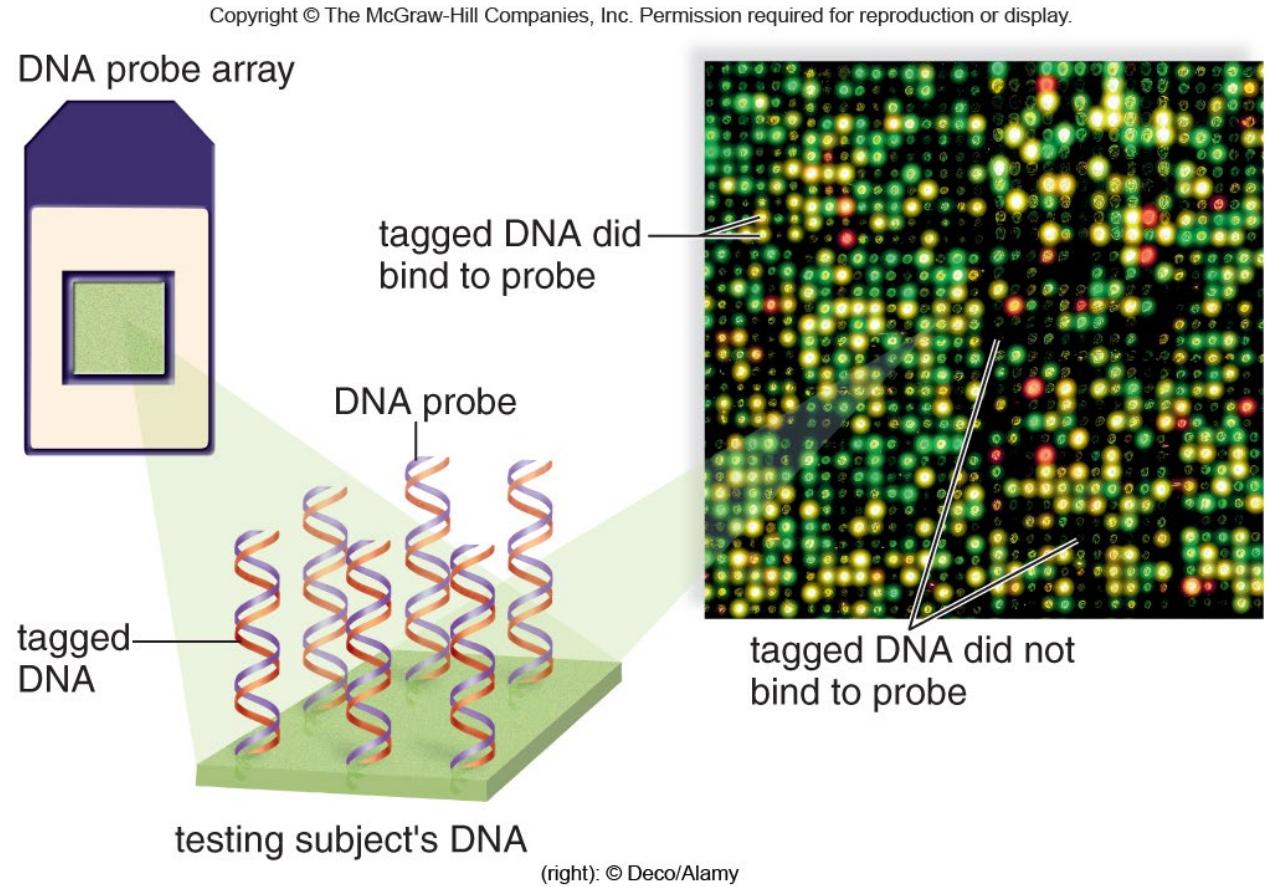
DNA Chips

- Contains up to thousands of DNA fragments arranged on a glass plate or slide
- Also known as DNA microarray
 - To analyse genetic profile of individuals
 - To study expression of thousands of genes at one time
 - To screen for genetic abnormalities, pathogens, or cancer

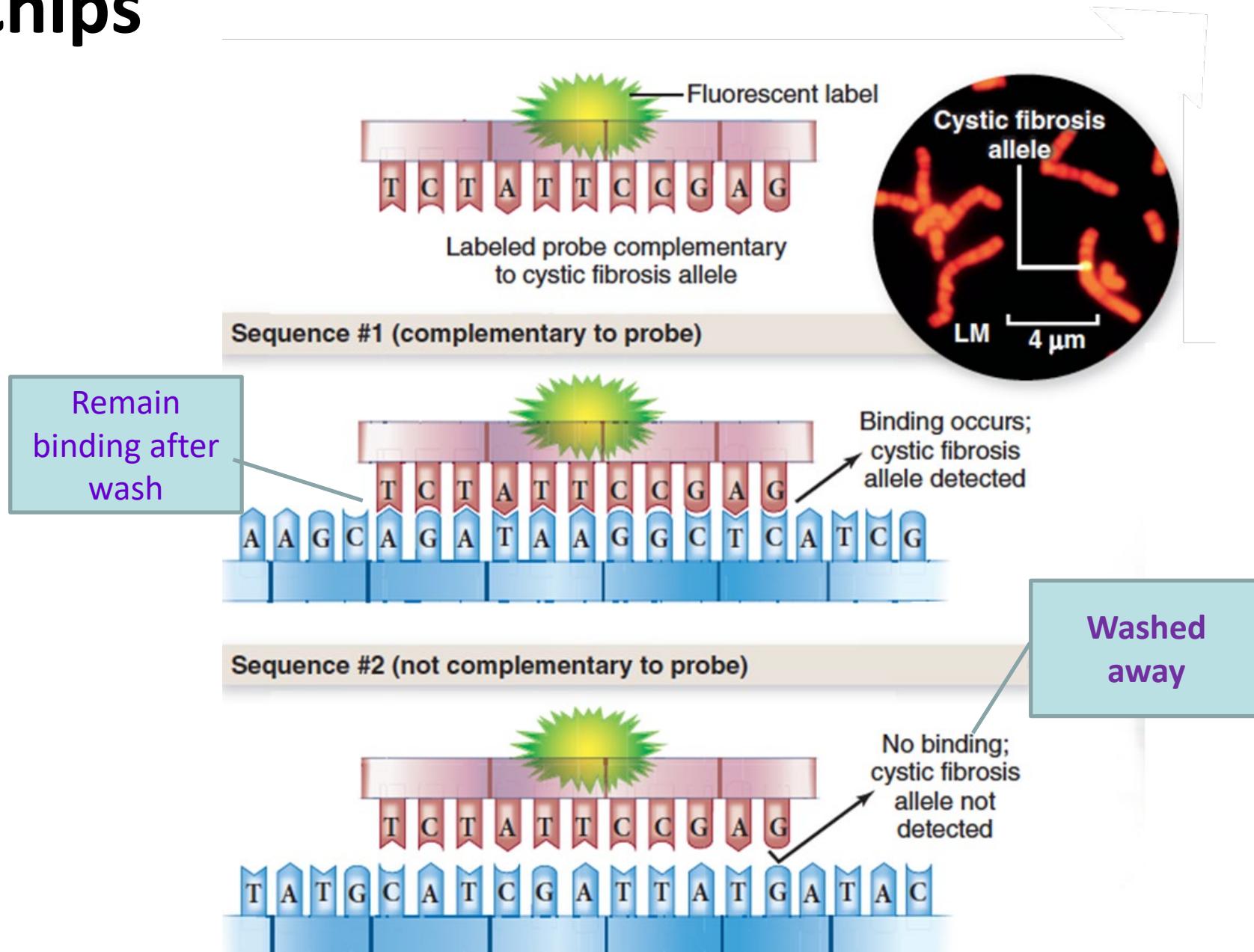


DNA Chips

- An individual's mRNA or genomic DNA only basepairs (or hybridises) with DNA spots on chip with complementary sequences
 - Probes reveal where mRNA or genomic DNA has hybridised

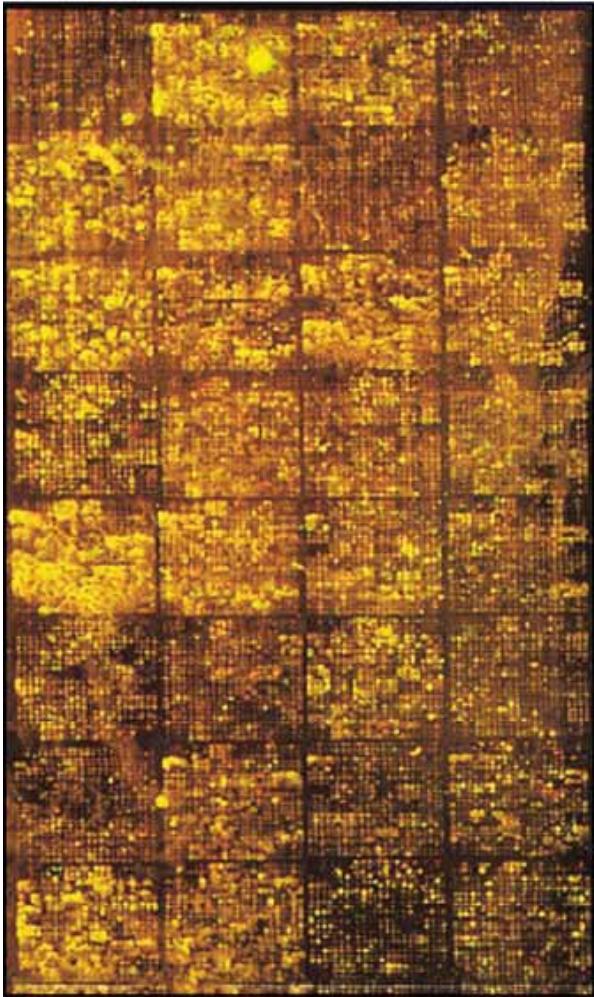


DNA Chips

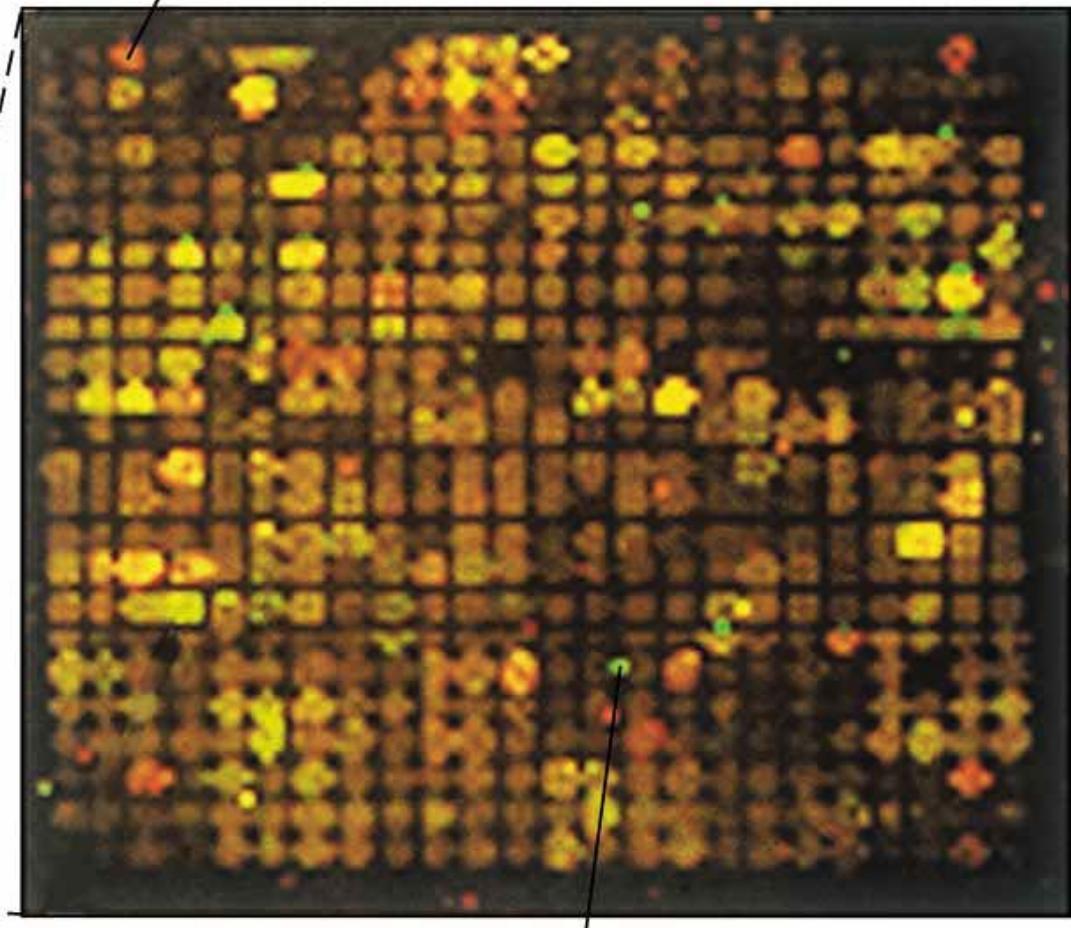


DNA Chips

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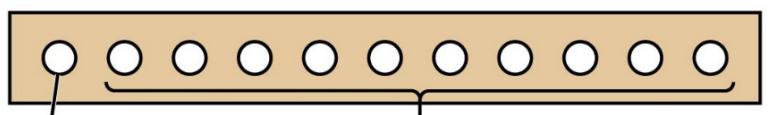


Red spots reveal genes with
higher expression in high-grade tumor

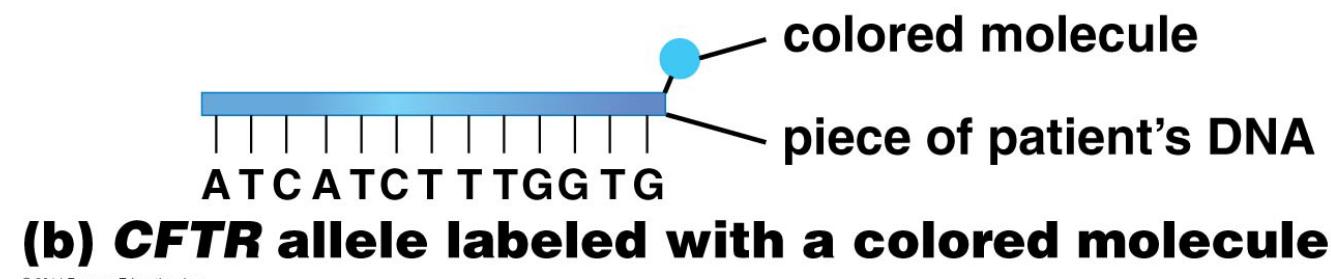


Genetic Screening

- Diagnostic arrays can be used to predict probability of a child with inherited genetic disorders
 - Prenatal diagnosis – embryo or foetus tested before birth
- Cystic fibrosis
 - Disease caused by any of 1,500 possible defective *CFTR* alleles (versions of a gene)
 - Only 32 alleles are common

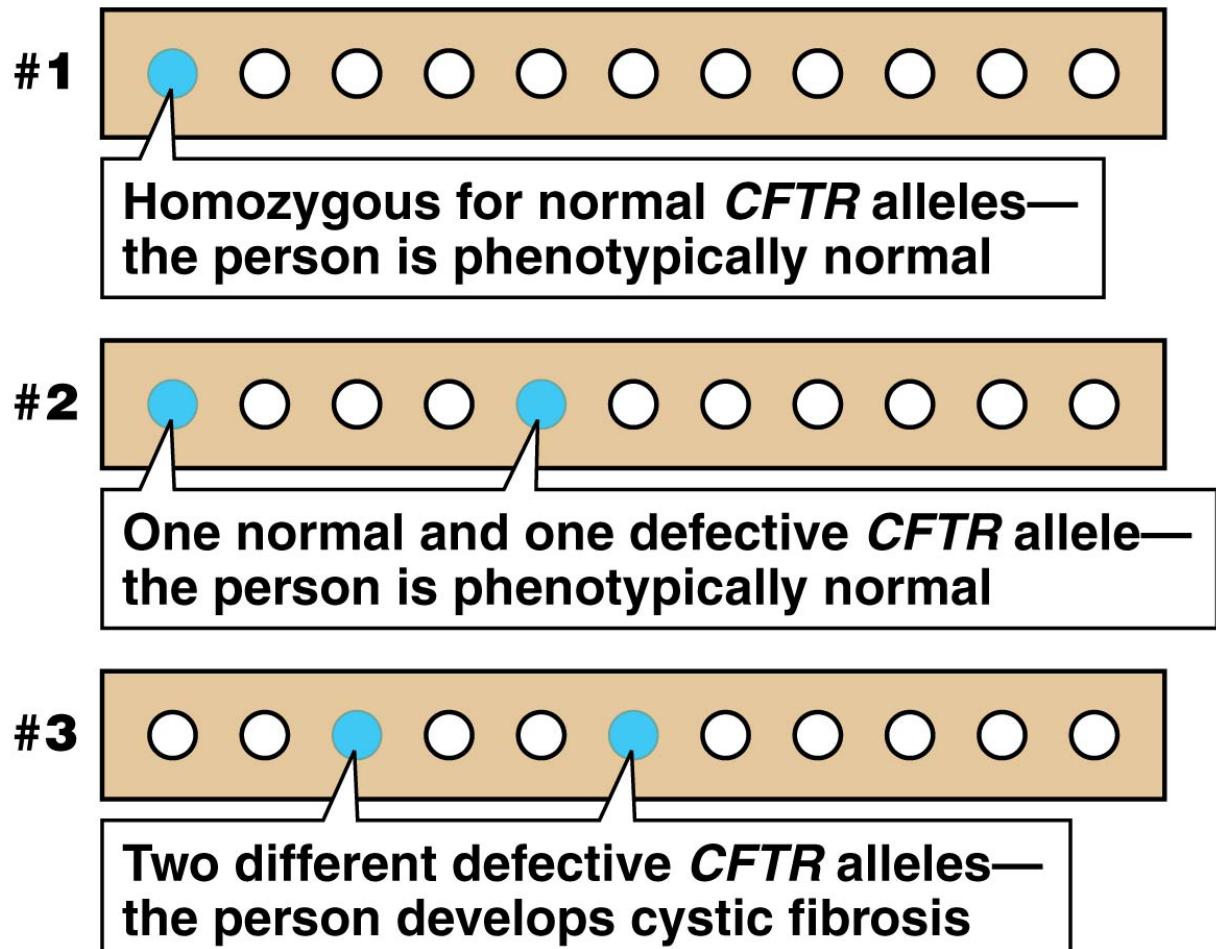


(a) Linear array of probes for cystic fibrosis



Genetic Screening

- Cystic fibrosis diagnostic arrays
 - Contain a specific DNA probe for each of a number of common *CFTR* alleles
 - DNA sample is cleaved, separated into single-stranded DNA, and labelled with coloured molecule
 - Array then bathed with labelled DNA sample solution and scanned for which *CFTR* allele the DNA sample binds to



(c) Linear arrays with labeled DNA samples from three different people

Case Study: Prenatal Genetic Screening

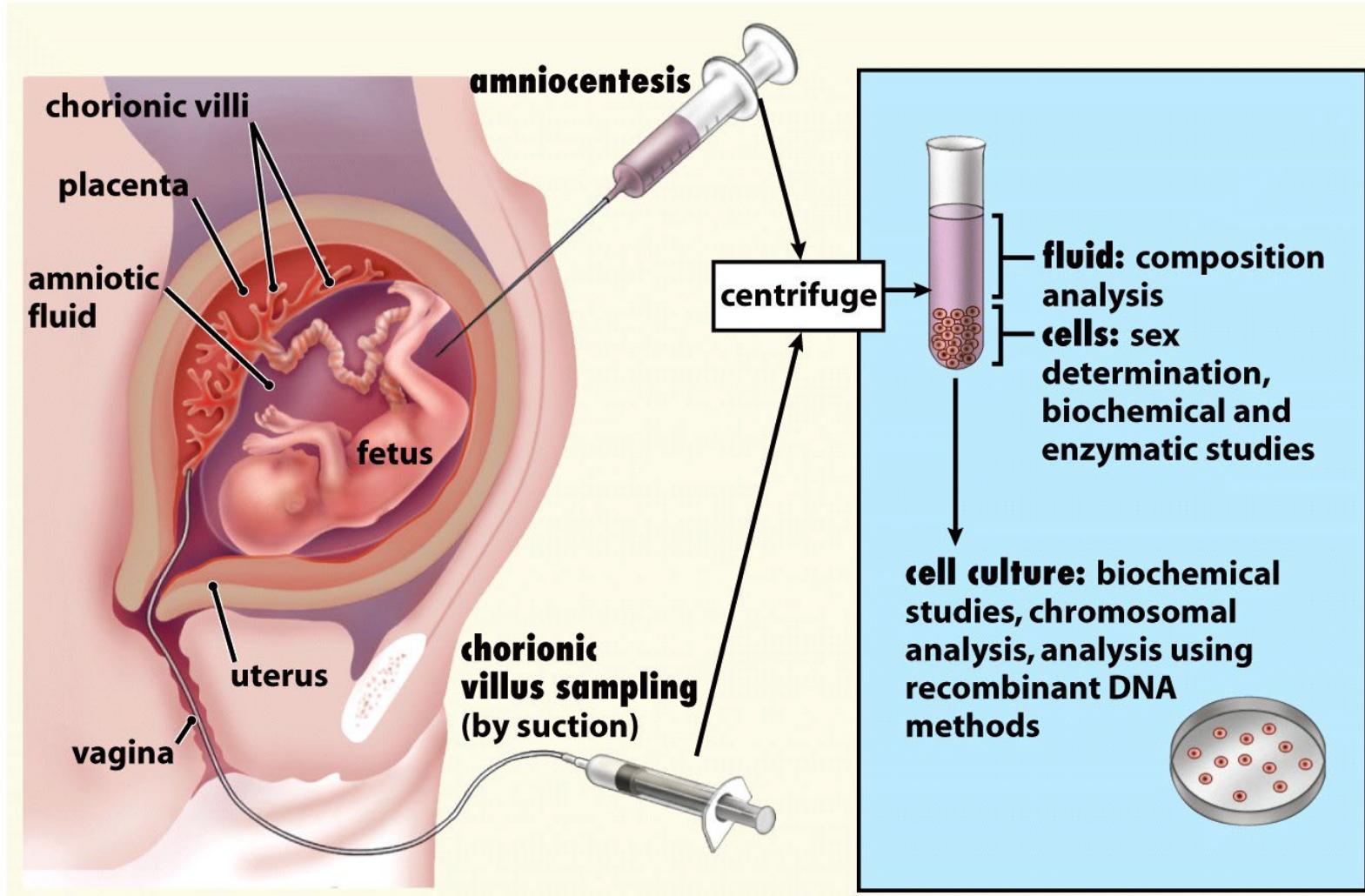


Figure E13-5 Biology: Life on Earth, 8/e
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DNA Barcoding



- What sushi are you really eating?
- Mislabeled seafood in markets in SG
- How can DNA Barcoding stop illegal wildlife trafficking?
 - Identifying the species of origin of meat, skin, feathers is often difficult - DNA barcoding can solve this



Outline

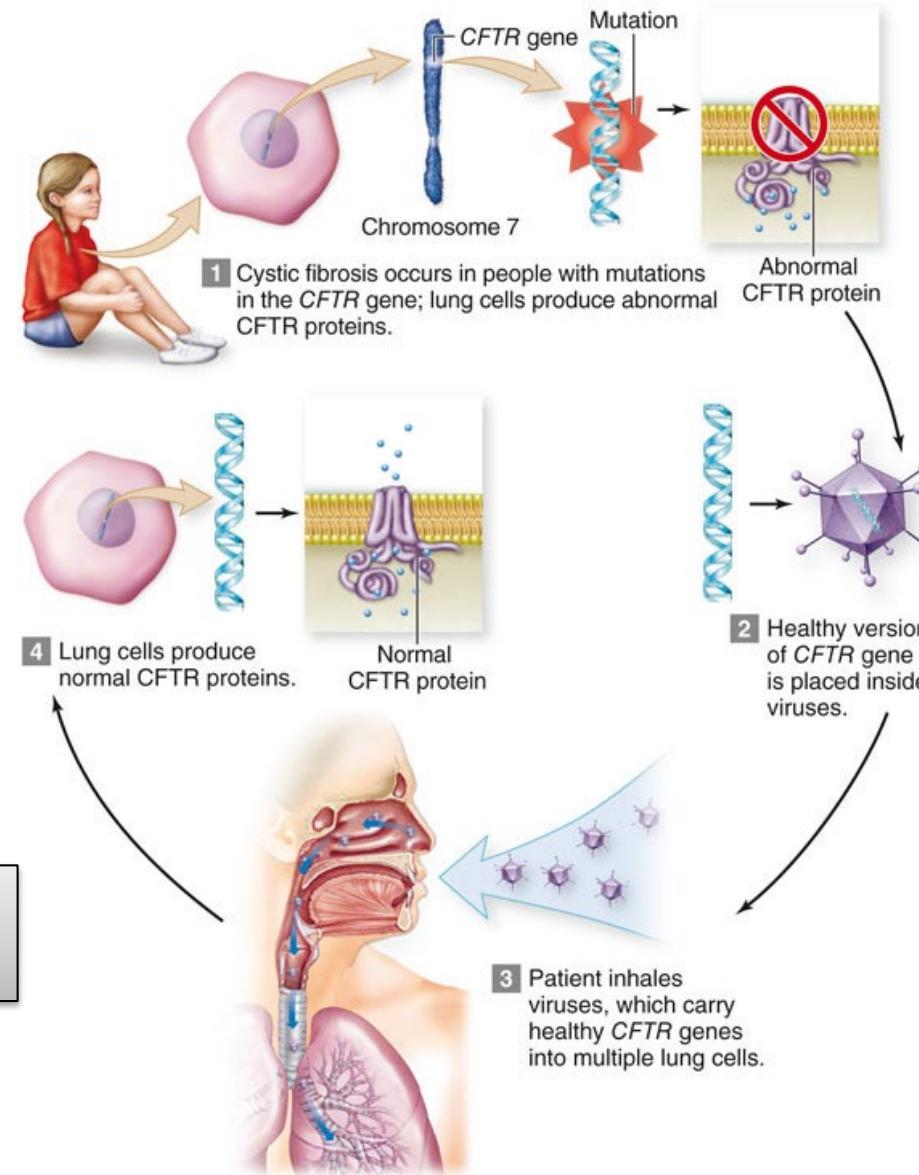
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 - Gel electrophoresis
- DNA probes
- DNA chips
- Genetic screening
- Gene Therapy
- Issues

Gene Therapy

- Insertion of healthy genes into cells to treat genetic disorders
- Viruses or phospholipid vesicles used but very challenging
 - Must be directly delivered to correct cell type
 - Must not alert immune system
 - Must express inserted genes
- Being tested as treatment for several types of cancer, sickle-cell anaemia, cystic fibrosis, and other inherited diseases

Gene Therapy Replaces Faulty Genes

Gene therapy may someday provide new treatment options for genetic diseases by replacing a faulty gene in a person's cells.



Gene therapy

- X-linked severe combined immunodeficiency disease (SCID-X1)
 - Caused by mutated allele of *IL2RG* gene on chromosome
 - Children with SCID-X1 can survive only in germ-free isolation tents

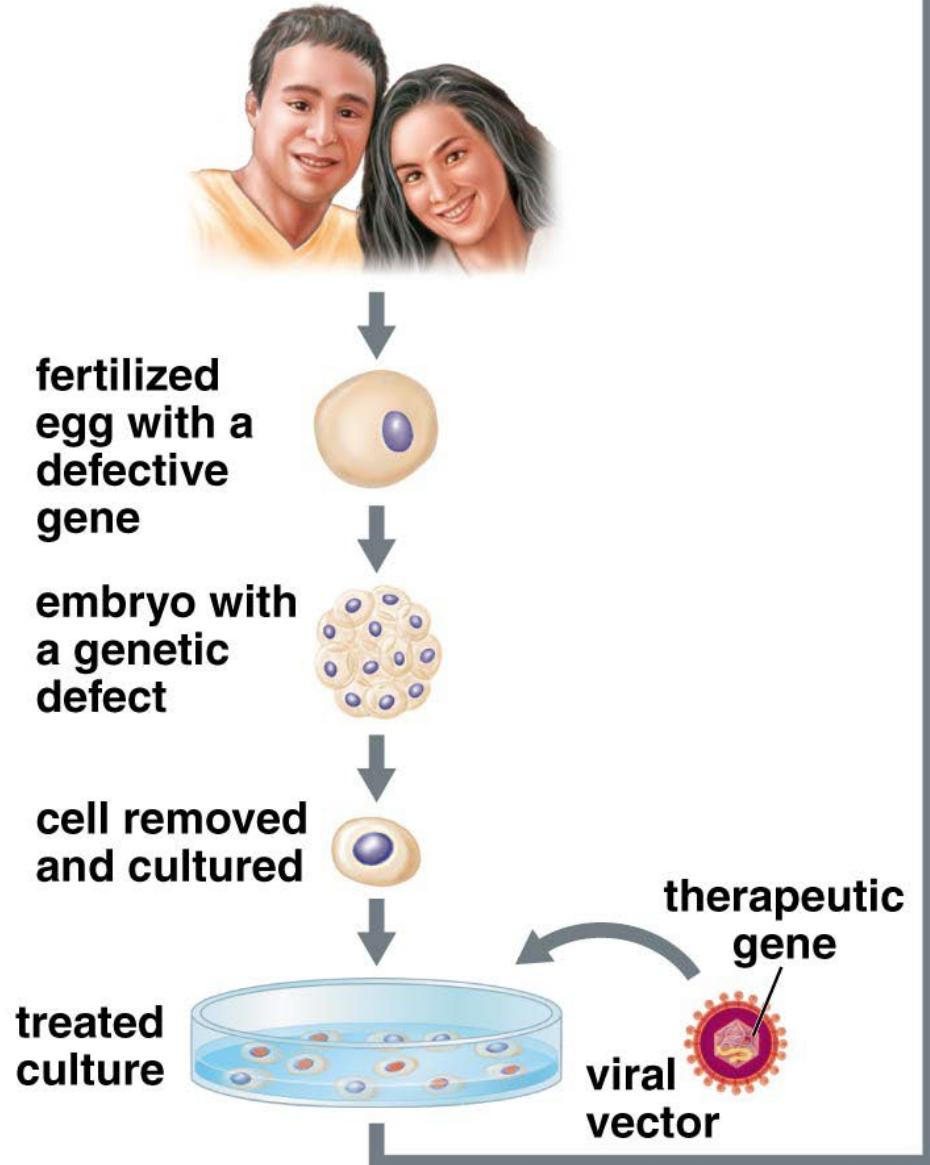


OpenStax Microbiology

Gene therapy

- In 1998, virus used to insert healthy alleles of gene into bone marrow stem cells of 20 boys with SCID-X1
 - Within months 18 boys left their isolation tents for good, with their immune systems repaired
 - However, 5 of the 20 boys developed leukaemia and one of them has died –probably because virus-inserted gene had disrupted control of cell division
- In 1999, in a separate study, severe allergic reactions to viral vector resulted in death

parents with a genetic disease



genetically corrected cell from culture

genetically corrected egg cell

genetically corrected clone of the original embryo

egg cell without a nucleus

healthy baby

Issues

- **Safety issues**
 - Could ingestion of Bt protein in insect-resistant plants be dangerous to humans?
 - Are transgenic fish producing extra growth hormone dangerous to eat?
 - Could GM crops cause allergic reactions?
- **Legal issues**
 - Who has access to my genetic information?
 - Should animals be modified to provide organs for human transplants?

Issues

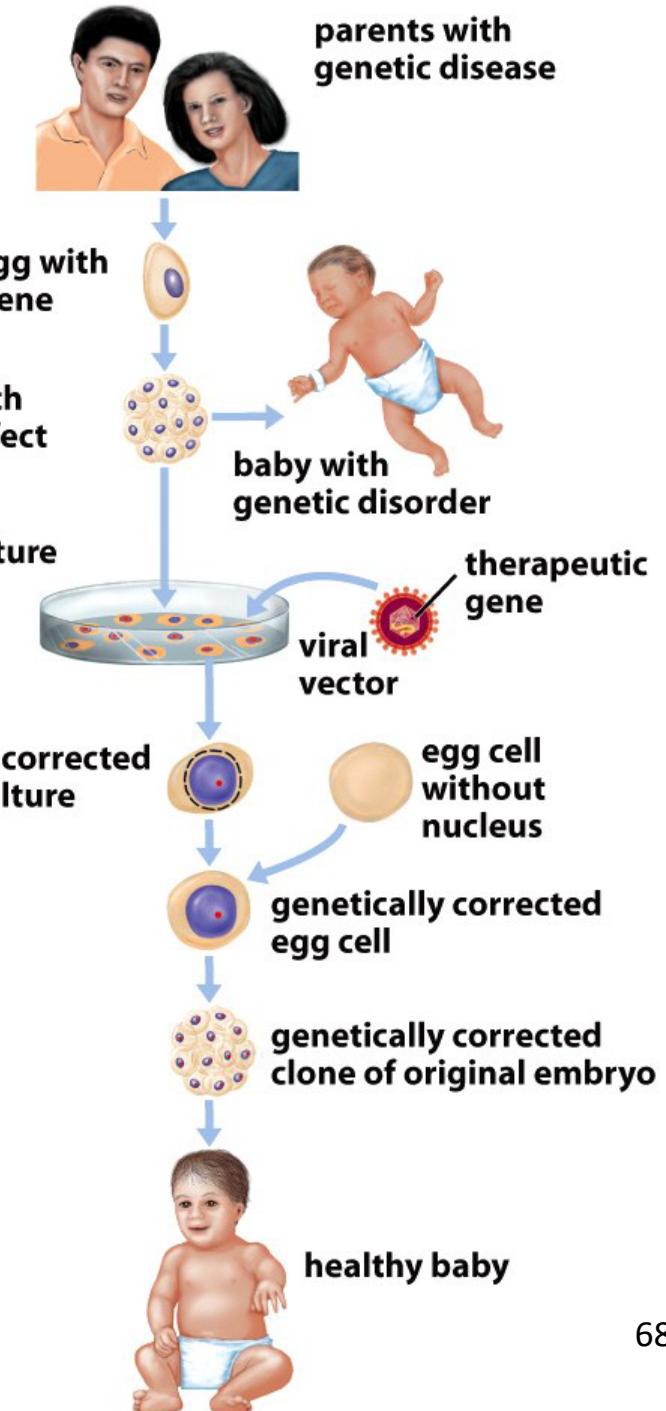
- **Environmental issues**

- Could herbicide resistance genes be transferred to weed species creating superweeds?
- Could GM fish reduce biodiversity in wild population if they escape?
 - Reduced diversity in wild makes population of fish more susceptible to catastrophic disease outbreaks



Ethical issues

- Should parents be given information about the genetic health of an unborn foetus?
 - Should parents be allowed to design or correct the genomes of their offspring?
- *Who decides what should be “corrected”?*
 - Who gets well and who gets enhanced?
- Should humans be cloned?





When do we need human gene engineering?

(Modify genetic material of an organism, usually using recombinant DNA technology)

Case 1



Will you select genetic engineering to treat a gene defects in your baby?

Case 2

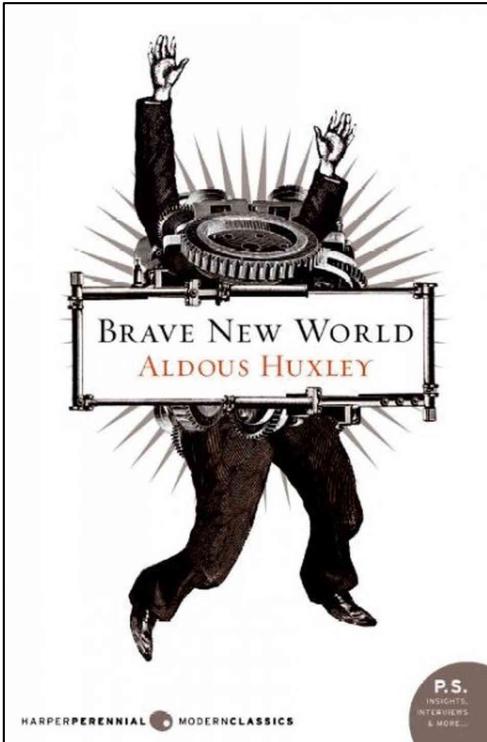


Genetically engineered super blood to boost your baby's physical strength?

Issues

One egg, one embryo, one adult – normality. But a bokanovskified egg will bud, will proliferate, will divide. From eight to ninety-six buds, and every bud will grow into a perfectly formed embryo, and every embryo into a full-sized adult. Making ninety-six human beings grow where only one grew before. Progress.

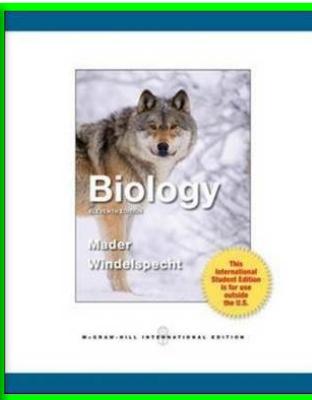
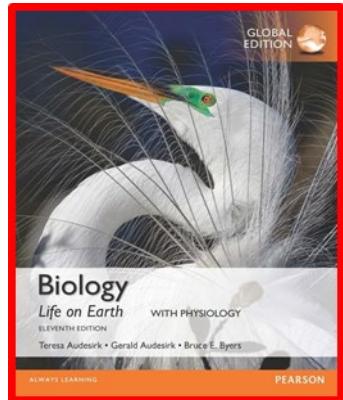
*Standard men and women; in uniform batches. The whole of a small factory staffed with the products of a single bokanovskified egg.
“Ninety-six identical twins working ninety-six identical machines!”*



Aldous Huxley, *Brave New World* (1932)

- First published, 1932
- NUS Central Library – PR6015 H986B

Text Books/References



This Lecture: Biotechnology

Chapters 14

Chapter 3, 7, 8

Chapters 13

Chapter 9, 10

~ The End of Entire Course ~

Reminder!

We value your online feedback

Try MCQs before the tutorial

Review: Chemistry of Life, Cell, Energy of life, DNA & Genes, Biotech; Summary and Make Connections

Thurs (7th April): 10am to 12pm over Zoom