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生科館 Rm730

# Chapter 12

## DNA Technology and Genomics

PowerPoint Lectures

***Campbell Biology: Concepts & Connections, 8th Edition, Global Edition***  
REECE • TAYLOR • SIMON • DICKEY • HOGAN

Lecture by Edward J. Zalisko

# Introduction

GMO safe?

- Hawaii's papaya industry seemed doomed just a few decades ago.
  - A deadly pathogen called the **papaya ringspot virus (PRV)** had spread throughout the islands.
  - It appeared poised to completely decimate the papaya plant population.
- Scientists from the University of Hawaii were able to rescue the industry by creating new, genetically engineered **PRV-resistant strains of papaya**.
- Today, the papaya industry is once again vibrant, and the vast majority (>75%) of Hawaii's papayas are **genetically modified organisms (GMOs)**.

銷售須標示影響購買意願  
台灣？有實驗品種但目前禁止商業種植  
偷種與混雜

輪點病毒



- Some critics have raised safety concerns
  - for the people who **eat** them and for the **environment**.
- Should we in fact be concerned about the **safety** of GMO crops?
  - This question continues to foster considerable debate and disagreement.
- In addition to GMOs in our diet, **DNA technologies** affect our lives in many other ways.
  - Gene cloning is used to produce medical and industrial products.
  - DNA profiling has changed the field of forensic science.
  - New technologies produce valuable data for biological research.
  - DNA can be used to investigate historical questions.

- DNA technology
  - has rapidly revolutionized the field of forensics,
  - permits the use of gene cloning to produce medical and industrial products,
  - allows for the development of genetically modified organisms for agriculture,
  - permits the investigation of historical questions about human family and evolutionary relationships, and
  - is invaluable in many areas of biological research.



## DNA重新鑑定，陳龍綺含冤1490日終獲無罪 <http://www.coolloud.org.tw/node/78352>

陳龍綺先生於2010年涉入一場妨害性自主官司，歷經1490個含冤待雪的日子，終在4月11日無罪確定，是繼江國慶後，第一位於有罪判決確定後，經DNA重新鑑定證明無辜，案件再審重獲清白者。同時，也是台灣以最新23組Y染色體STR之DNA鑑定的首例，亦是冤獄平反協會2012年成立以來第一件平反案例。

## 北市內湖女裸屍案再審 呂介閔逆轉改判無罪

<https://tw.appledaily.com/new/realtime/20151230/764010/>

命案時間：2000.7；定讞：2010，13年徒刑；證據：咬痕、精液

要求再審：2014（已服刑4年）；2015.12 無罪釋放

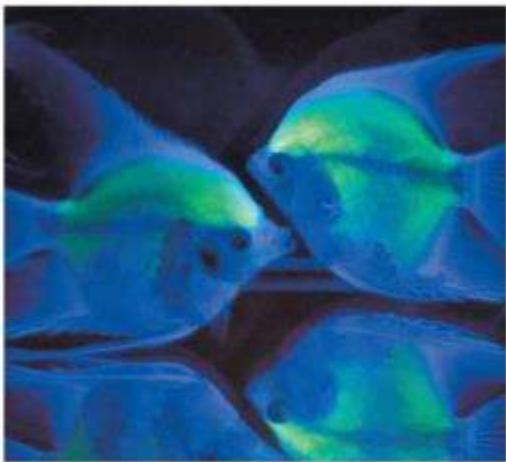
新事證：微量男性唾液經新技術鑑定（男性Y染色體DNA-STR型別鑑定方法），非被告所留。

## 被告可聲請重驗DNA避免冤案-立法院三讀通過刑事案件確定後去 氧核醣核酸鑑定條例 <http://www.lawbank.com.tw/news/NewsContent.aspx?NID=139257.00>

2016.11.16公告「刑事案件確定後去氧核醣核酸鑑定條例」，也就是DNA鑑定條例，未來刑事案件判決定讞後，受判決人及其親屬等得向法院聲請DNA重新鑑定，聲請法院重新判決，以排除冤案，開啟再審可能。立法院指出，過去要翻案必須依刑事訴訟法第420條第1項第6款提出新證據才能聲請再審，但DNA檢體在政府機關保管下，僅法官、檢察官有權重驗證據，被告無法聲請重驗，更難啟動再審，導致企圖翻案的被告求助無門；未來可以透過最新的DNA鑑定技術，讓過去因DNA鑑定不足或有誤的冤案能有平反的機會，若受判決人已死亡，也可由相關家屬提出申請。

Figure 12.0\_1

## Chapter 12: Big Ideas



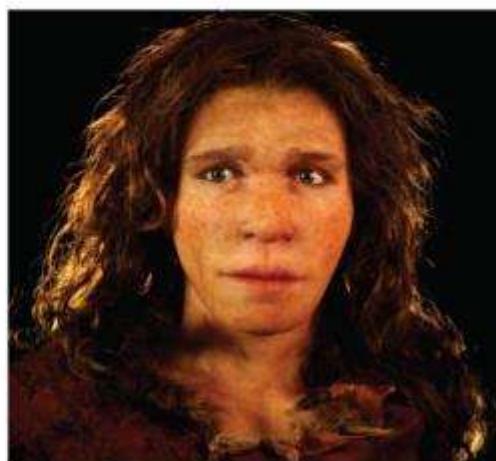
**Gene Cloning**  
**12.1-12.5**



**Genetically Modified  
Organisms**  
**12.6-12.10**



**DNA Profiling**  
**12.11-12.16**

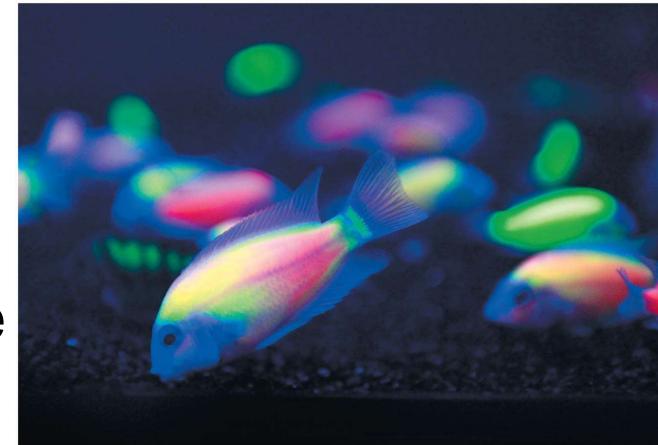


**Genomics**  
**12.17-12.21**

# **GENE CLONING**

## 12.1 Genes can be cloned in recombinant plasmids

- Biotechnology is the manipulation of organisms or their components to make useful products.
- For thousands of years, humans have
  - used microbes to make wine and cheese and
  - selectively bred stock, dogs, and other animals.
  - DNA technology is the set of modern techniques used to study and manipulate genetic material.

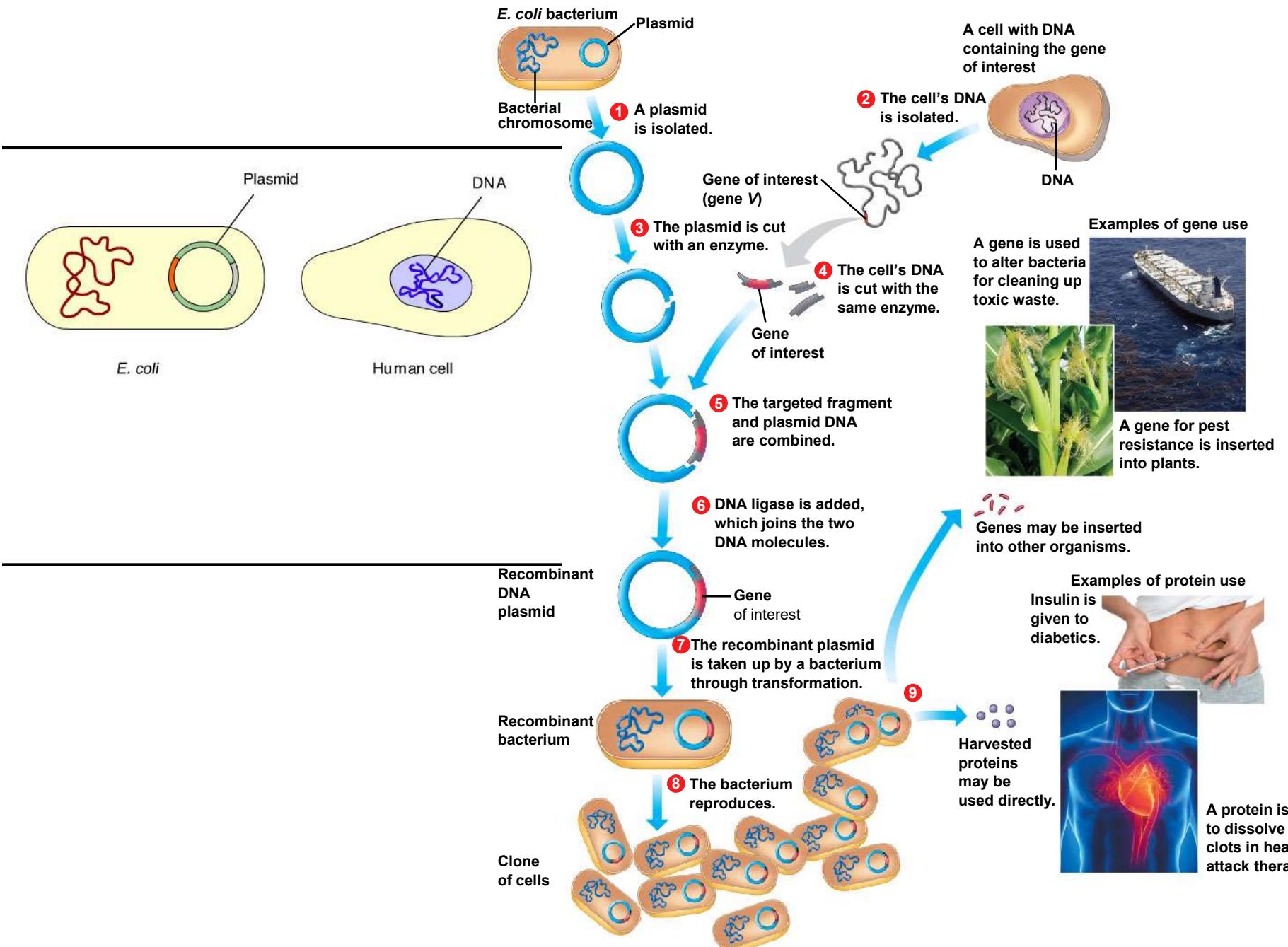


## 12.1 Genes can be cloned in recombinant plasmids

- Genetic engineering involves manipulating genes for practical purposes.
  - Gene cloning leads to the production of multiple, identical copies of a gene-carrying piece of DNA in vitro (in a test tube) to form a single DNA molecule.
  - Recombinant DNA is formed by joining nucleotide sequences from two different sources. and often different species.
    - One source contains the gene that will be cloned.
    - Another source is a gene carrier, called a **vector**.
    - Plasmids (small, circular DNA molecules 載體  
質體 independent of the bacterial chromosome) are often used as vectors.

## 12.1 Genes can be cloned in recombinant plasmids

- Steps in cloning a gene
  1. Plasmid DNA is isolated.
  2. DNA containing the gene of interest is isolated.
  3. Plasmid DNA is treated with a restriction enzyme that cuts in one place, opening the circle.
  4. DNA with the target gene is treated with the same enzyme and many fragments are produced.
  5. Plasmid and target DNA are mixed and associate with each other.
  6. Recombinant DNA molecules are produced when **DNA ligase** joins plasmid and target segments together.
  7. The recombinant plasmid containing the target gene is taken up by a bacterial cell.
  8. The bacterial cell reproduces to form a **clone**, a group of genetically identical cells descended from a single ancestral cell.



## 12.2 Enzymes are used to “cut and paste” DNA

限制酶

- **Restriction enzymes** cut DNA at specific sequences.
  - Each enzyme binds to DNA at a different **restriction site**.
  - Many restriction enzymes make staggered cuts that produce **restriction fragments** with single-stranded ends called “sticky ends.”
  - Fragments with complementary sticky ends can associate with each other, forming recombinant DNA.
- **DNA ligase** joins DNA fragments together.

**Figure 12.2-5**

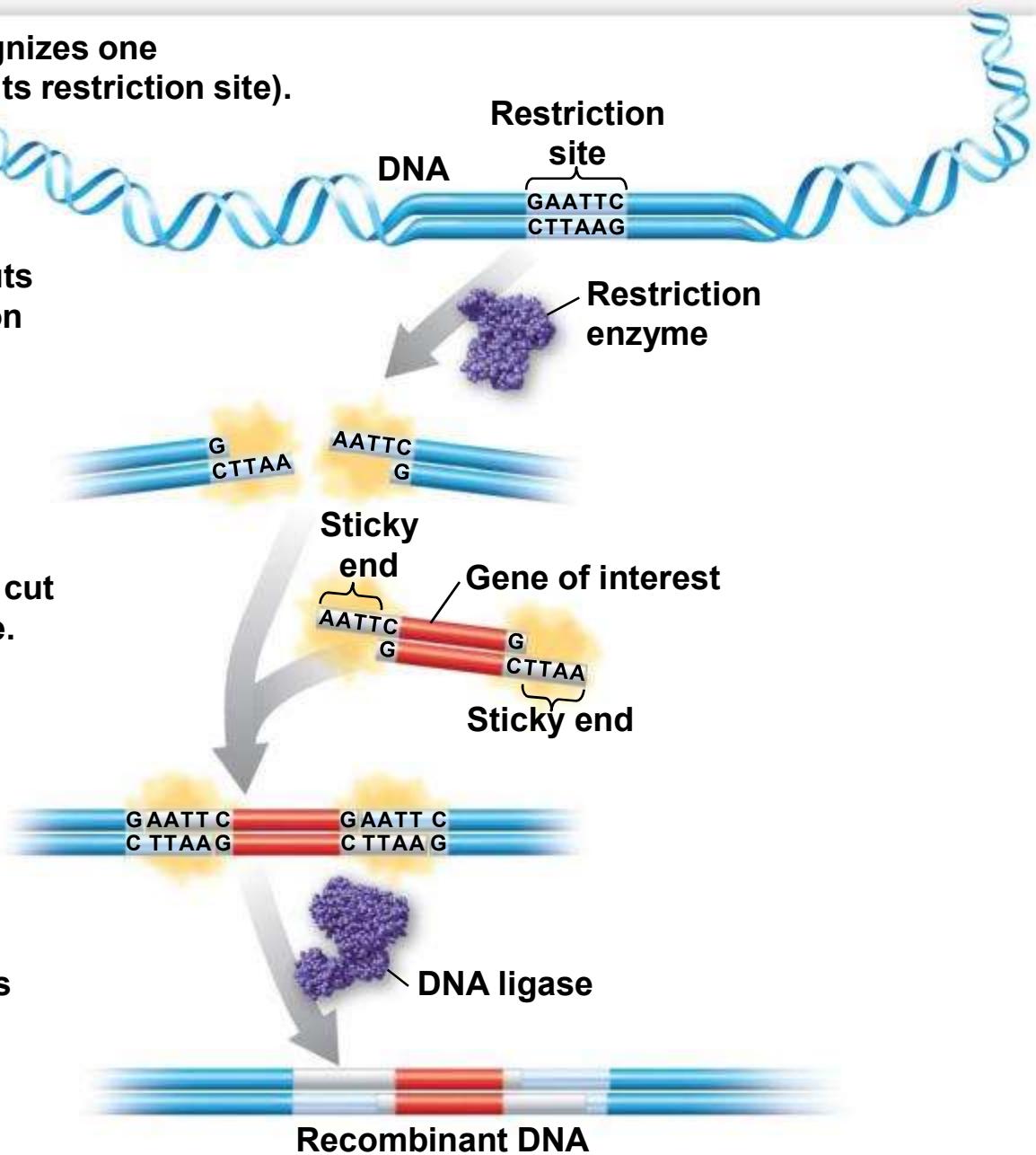
**Every restriction enzyme recognizes one specific nucleotide sequence (its restriction site).**

**A restriction enzyme always cuts DNA sequences at its restriction site in an identical manner.**

**A piece of DNA from another source (the gene of interest) is cut by the same restriction enzyme.**

**The DNA fragments from the two sources stick together by hydrogen bonding of base pairs.**

**The enzyme DNA ligase creates new covalent bonds that join the backbones of the DNA strands. The result is a piece of recombinant DNA.**



# The Nobel Prize in Physiology or Medicine 1978



Werner Arber  
Prize share: 1/3

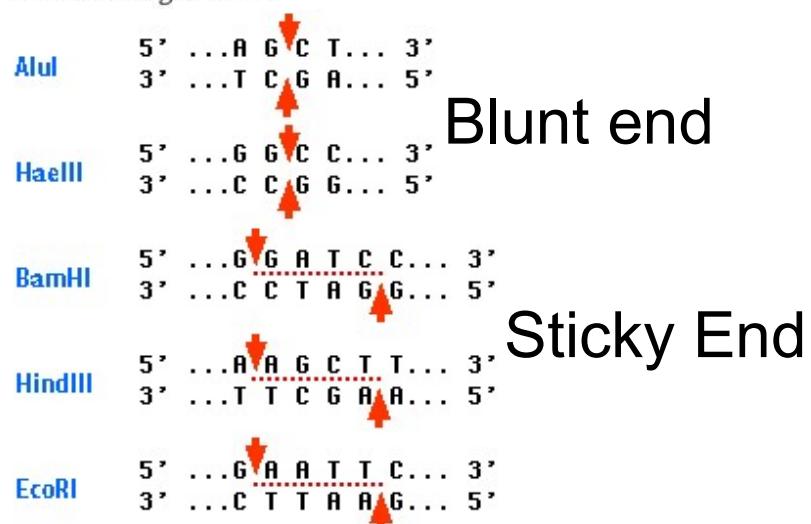


Daniel Nathans  
Prize share: 1/3



Hamilton O. Smith  
Prize share: 1/3

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

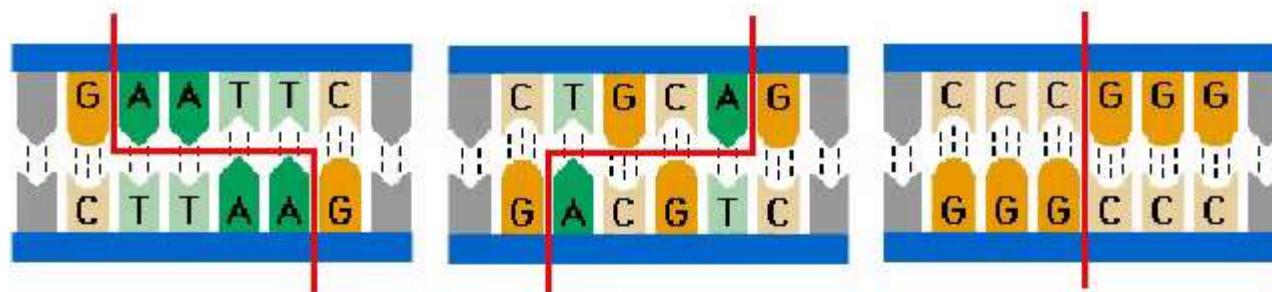


AluI and HaeIII produce blunt ends

BamHI, HindIII and EcoRI produce "sticky" ends [users.rcn.com](http://users.rcn.com)

Restriction enzymes are found in many different strains of **bacteria** where their biological role is to participate in **cell defense**. These enzymes "restrict" foreign (e.g. viral) DNA that enters the cell, by destroying it. The host cell has a restriction-modification system that **methylates its own DNA** at sites specific for its respective restriction enzymes, thereby protecting it from cleavage. Over 800 known enzymes have been discovered that recognize over 100 different nucleotide sequences.

<https://www.thebalance.com/what-are-restriction-enzymes-375674>



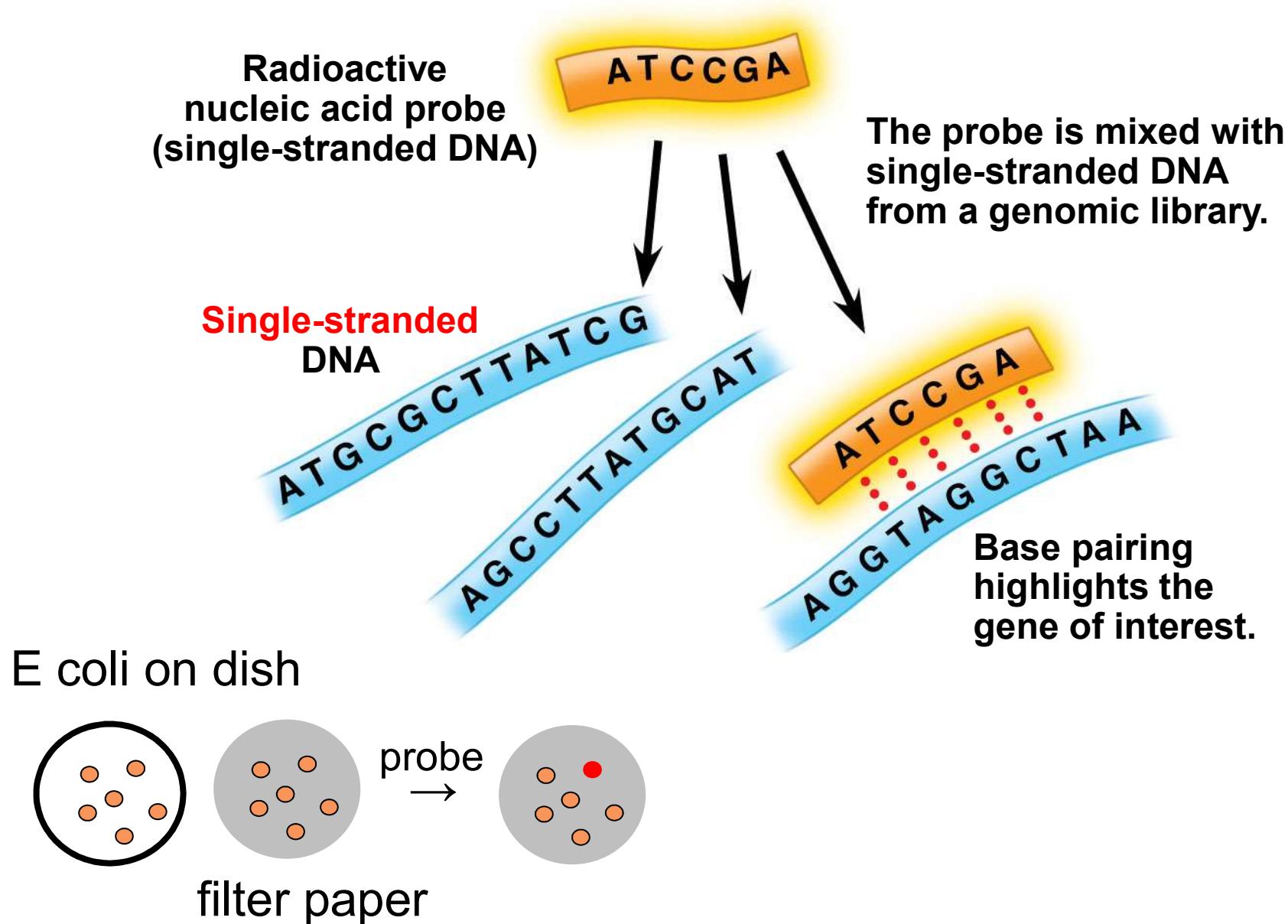
Sticky End  
Blunt end

Animation: Restriction Enzymes  
Right click on animation / Click play

## 12.3 Nucleic acid probes can label specific DNA fragments

- **Nucleic acid probes** bind very selectively to cloned DNA. 探針
  - **Probes** can be DNA or RNA sequences **complementary** to a portion of the **gene of interest**.
  - A probe binds to a gene of interest by base pairing.
  - Probes are labeled with a radioactive isotope or fluorescent tag for detection.
- One way to screen a gene library is as follows:
  1. Bacterial clones are transferred to filter paper.
  2. Cells are broken apart and the DNA is separated into single strands.
  3. A probe solution is added and any bacterial colonies carrying the gene of interest will be tagged on the filter paper.
  4. The clone carrying the gene of interest is grown for further study.

Figure 12.5

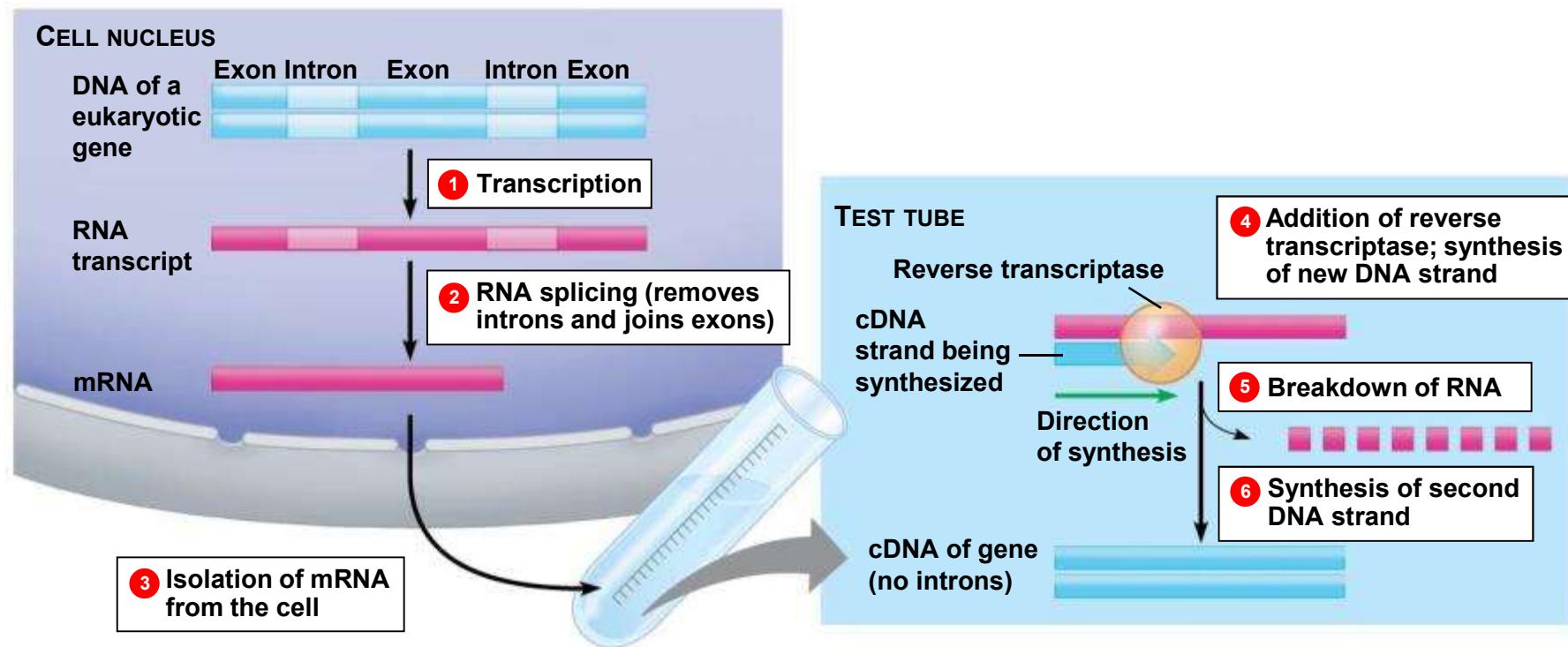


## 12.4 Reverse transcriptase can help make genes for cloning

- A researcher can focus on the genes expressed in a particular kind of cell by using its mRNA as the starting material for cloning.
- In this process,
  - mRNA from a specific cell type is the template,
  - **reverse transcriptase** produces a DNA strand from mRNA,
  - DNA polymerase produces the second DNA strand, and
  - the DNA that results from such a procedure, called **complementary DNA (cDNA)**, represents only the subset of genes that had been transcribed into mRNA in the starting cells.

互補

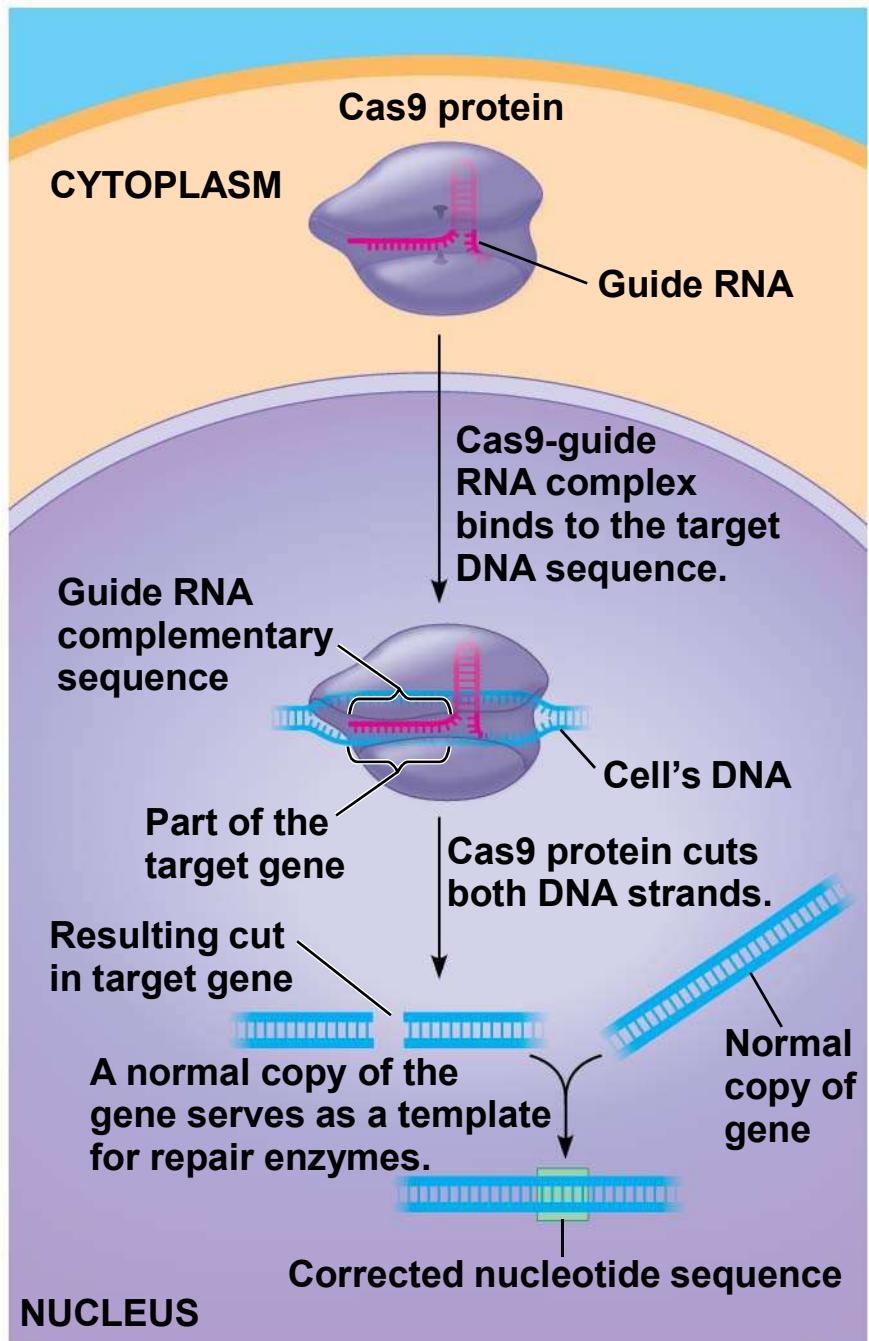
Figure 12.4



- Advantages of cloning with cDNA include the ability to study genes responsible for specialized characteristics of a particular cell type and obtain gene sequences that are smaller in size, easier to handle, and **do not have introns**.

## 12.5 New techniques allow a specific gene to be edited

- The CRISPR-Cas9 system allows researchers to target a specific gene in a living cell for removal or editing.
- Unlike restriction enzyme which cut only a particular sequence; Cas9 will cut any sequence to which it is directed by a molecule of RNA.
- Introducing a **Cas9-guide RNA complex** into a cell: the RNA is complementary to a target DNA sequence; after Cas9 cut the both strands, DNA repair enzymes randomly insert nucleotides, usually rendering the gene nonfunctional.
- Highly successful to “knock-out” a given gene
- For Crispr-Cas9 system, a segment of gene (DNA) was introduced along with the Cas9/guide RNA complex as the template for repairmen to “edit” the gene.
- 2014, researchers alter a faulty gene in a mouse embryo; 2015 mouse infected with virus to modify the faulty gene in muscles.



## 1. Patent war

<https://udn.com/news/story/6871/3402588>

<https://pansci.asia/archives/149097>

## 2. Ethical issues



日本科學家要求為基因編輯技術踩下煞車

日經中文網 - 2019年4月28日

禁止類似在中國誕生的「基因組編輯嬰兒」那樣操控生命及助長優生思想的 ... 由於被用於基因編輯嬰兒的「CRISPR-Cas9」技術的出現，人們能夠以 ...

使用前讓它更安全：基因神剪CRISPR 還有哪些問題待解？

PanSci 泛科學 (新聞發布) - 22 小時前

基因編輯寶寶事件」是中國生物學家賀建奎和他的團隊，利用CRISPR ... 理想狀況下，當Cas9 剪開DNA 時，會有機會更改DNA，但細胞願不願意將 ...



打破醫學道德界線，賀建奎列時代雜誌百大人物

科技新報 TechNews - 2019年4月18日

賀建奎使用CRISPR-Cas9 基因編輯技術修改人類胚胎，此技術是由夏彭提耶（Emmanuelle Charpentier）、道納（Jennifer A. Doudna）、張鋒等人 ...

爭議人物賀建奎入選全球最具影響力百大人物

多維新闻网 (新聞發布) - 2019年4月19日

查看全部



植入人類基因的猴子變聰明了嗎？中國科學家「基因轉換」又惹議

The News Lens 關鍵評論網 - 2019年4月12日

中國在2016年利用敲除關鍵基因的「CRISPR-cas9」方法，敲除了猴胚胎中調控 ...

賀建奎用基因編輯的方式，產生了2名據稱能抵抗愛滋病毒的嬰兒。

## 3. New animal models for diseases

# **GENETICALLY MODIFIED ORGANISMS**

## 12.6 Recombinant cells and organisms can mass-produce gene products

- Recombinant cells and organisms constructed by DNA technologies are used to manufacture many useful products, chiefly proteins.
- Bacteria are often the best organisms for manufacturing a protein product because bacteria
  - have plasmids and phages available for use as gene-cloning vectors,
  - can be grown rapidly and cheaply,
  - can be engineered to produce **large amounts** of a particular protein, and
  - often secrete the proteins directly into their growth medium.

## 12.6 Recombinant cells and organisms can mass-produce gene products

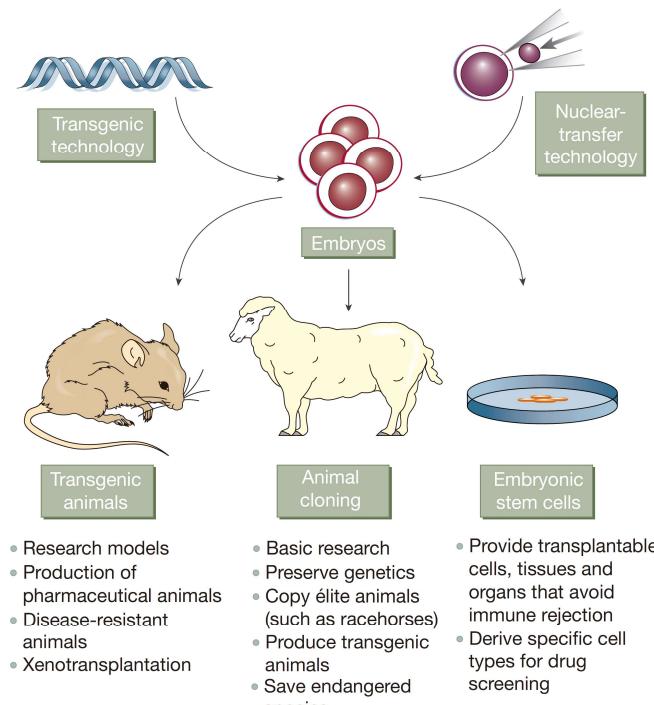
- Yeast cells 酵母菌
  - are eukaryotes,
  - are easy to grow,
  - have long been used to make bread and beer,
  - can take up foreign DNA and integrate it into their genomes, and
  - are often better than bacteria at synthesizing and secreting eukaryotic proteins.
- Mammalian cells must be used to produce proteins with chains of sugars. Examples include 紅血球生成素 Ch23
  - human erythropoietin (EPO), which stimulates the production of red blood cells,
  - factor VIII to treat hemophilia, and
  - tissue plasminogen activator (TPA) used to treat heart attacks and strokes. 組織型纖維蛋白溶酶原激活物 TPA活化plasminogen (纖維蛋白溶酶原)，使其成為plasmin (纖維蛋白溶酶)，以溶解傷口血塊

TABLE 12.6

## SOME PROTEIN PRODUCTS OF RECOMBINANT DNA TECHNOLOGY

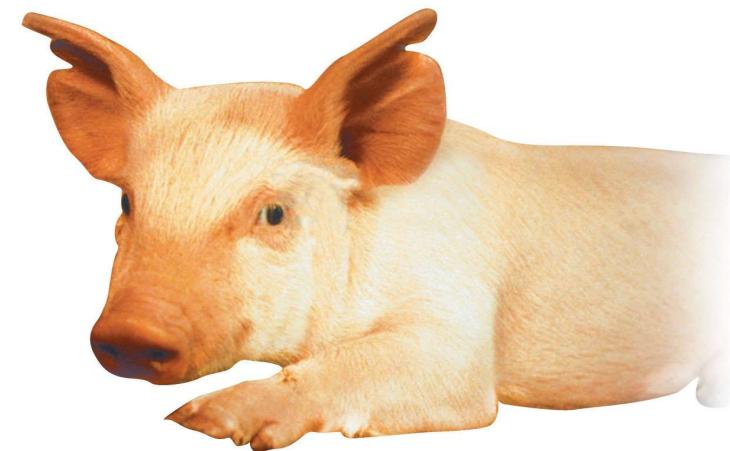
Product	Made by	Use
Human insulin	<i>E. coli</i>	Treatment for diabetes
Human growth hormone (HGH)	<i>E. coli</i>	Treatment for growth defects
Epidermal growth factor (EGF)	<i>E. coli</i>	Treatment for burns, ulcers
Interleukin-2 (IL-2)	<i>E. coli</i>	Possible treatment for cancer
Bovine growth hormone (BGH)	<i>E. coli</i>	Improving weight gain in cattle
Cellulase	<i>E. coli</i>	Breaking down cellulose for animal feeds
Taxol	<i>E. coli</i>	Treatment for ovarian cancer
Interferons (alpha and gamma)	<i>S. cerevisiae</i> , <i>E. coli</i>	Possible treatment for cancer and viral infections
Hepatitis B vaccine	<i>S. cerevisiae</i>	Prevention of viral hepatitis
Erythropoietin (EPO)	Mammalian cells	Treatment for anemia
Factor VIII	Mammalian cells	Treatment for hemophilia
Tissue plasminogen activator (TPA)	Mammalian cells	Treatment for heart attacks and some strokes

## The many uses of embryo technology.

*Nature* 428, 210-212 (11 March 2004)

**First “Pharm” Animals 2009**  
**antithrombin (抗凝血酶) in goats' milk.** GTC Biotherapeutics  
**2014 C1-esterase inhibitor in rabbit milk,** Salix Pharmaceuticals  
**2015 Sebelipase α in chicken egg,**  
**Alexion Pharmaceuticals**

- Pharmaceutical researchers are currently exploring the **mass production** of gene products by
  - whole animals or plants.
- **Recombinant animals**
  - are **difficult** and **costly** to produce and
  - must be cloned to produce **more** animals with the same traits.



## 12.7 DNA technology has changed the pharmaceutical industry and medicine

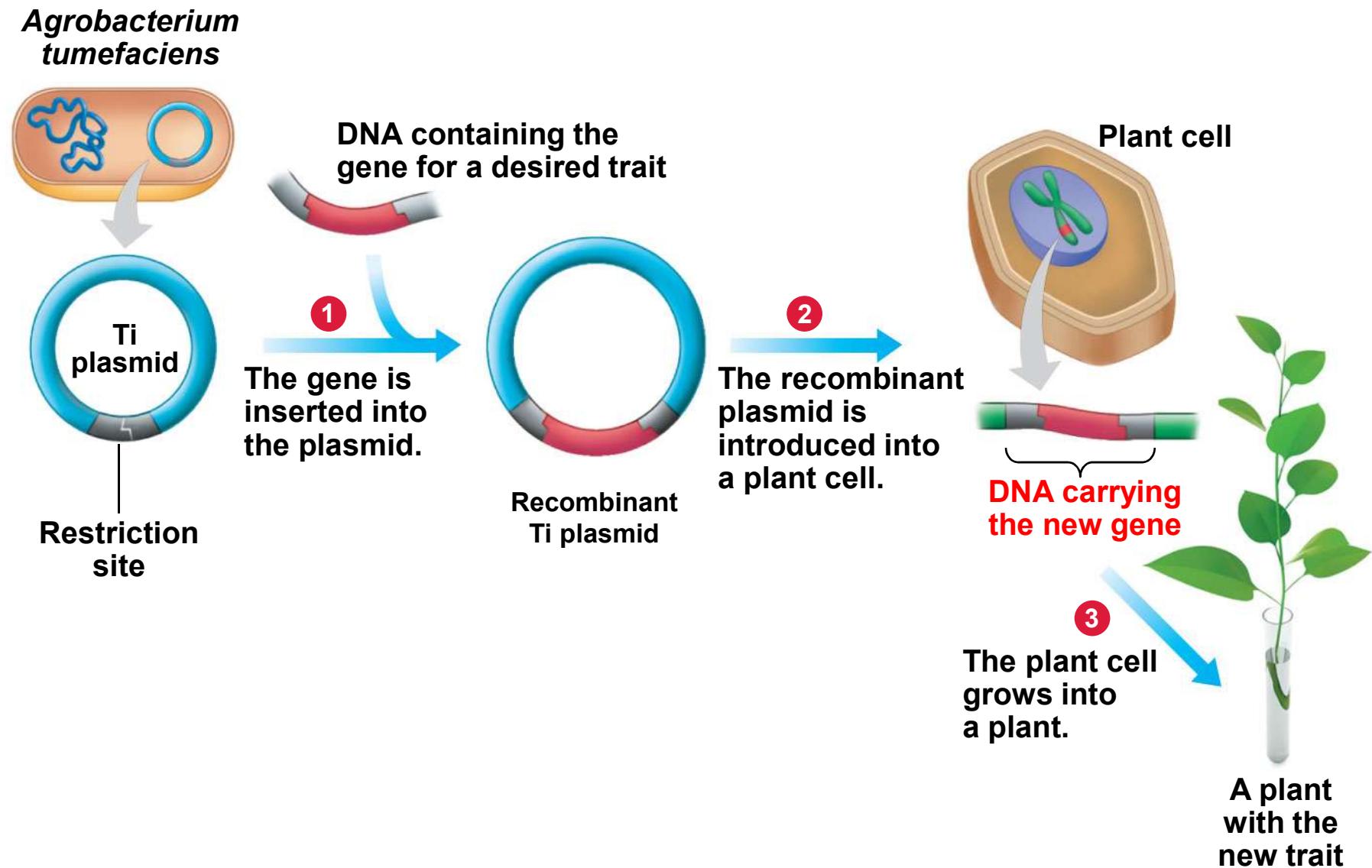
- DNA technology, including gene cloning, is widely used to produce medicines and to diagnose diseases.
- Therapeutic hormones produced by DNA technology include
  - insulin to treat diabetes and
  - human growth hormone to treat dwarfism.
  - tissue plasminogen activator (TPA), a protein that helps dissolve blood clots and reduces the risk of subsequent heart attacks.
  - DNA technology is used to
    - test for inherited diseases,
    - detect infectious agents such as HIV, and
    - produce **vaccines**, harmless variants (mutants) or derivatives of a pathogen that stimulate the immune system to mount a lasting defense against that pathogen, thereby preventing disease.



## 12.8 Genetically modified organisms are transforming agriculture

- Since ancient times, people have selectively bred agricultural crops to make them more useful.
- DNA technology is quickly replacing traditional breeding programs to improve the productivity of agriculturally important plants and animals.
- **Genetically modified organisms (GMOs)** contain one or more genes introduced by artificial means.
- Transgenic organisms contain at least one gene from another species.
- The most common vector used to introduce new genes into plant cells is a plasmid from the soil bacterium *Agrobacterium tumefaciens* called the **Ti plasmid**.

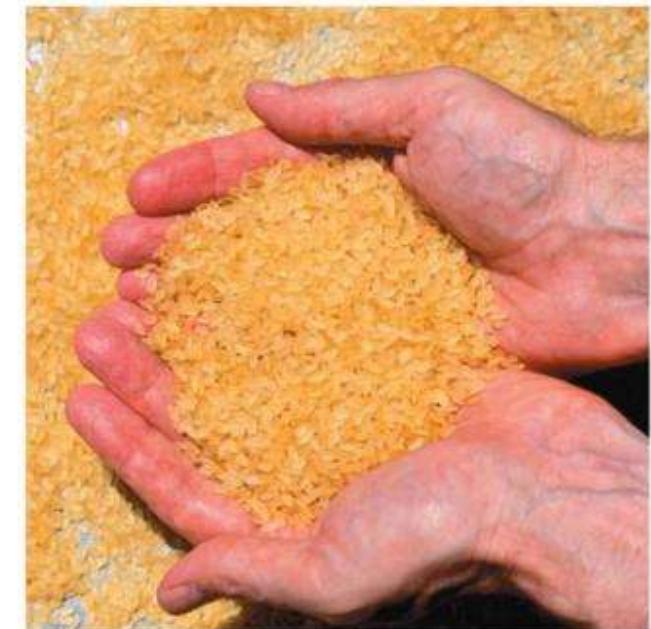
Figure 12.8A\_s3



© 2012 Pearson Education, Inc.

## 12.8 Genetically modified organisms are transforming agriculture

- GMO crops may be able to help a great many hungry people by improving
  - food production,
  - shelf life,
  - pest resistance, and
  - the nutritional value of crops.
- Golden Rice, a transgenic variety created in 2000 with a few daffodil genes, produces yellow grains containing beta-carotene, which our body uses to make vitamin A.
- Genetic engineers are now creating plants that make human proteins for medical use.



- Pharmaceutical trials currently under way involve using modified
  - rice to treat infant diarrhea, corn to treat cystic fibrosis, safflower to treat diabetes, and duckweed to treat hepatitis.
- Agricultural researchers are producing transgenic animals by injecting cloned genes directly into the nuclei of fertilized eggs.
  - Genetically modified pigs convert less healthy fatty acids to omega-3 fatty acids, producing meat with four to five times as much healthy omega-3 fat as regular pork.
  - Atlantic salmon, genetically modified to mature in half the time of conventional salmon and grow to twice the size.

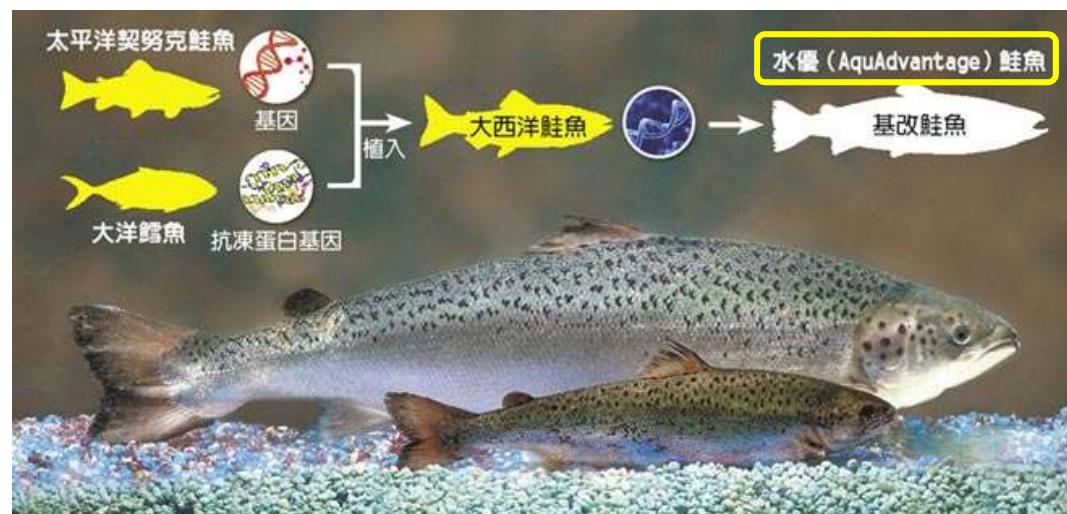
*Golden Rice* accumulates provitamin A ( $\beta$ -carotene) in the grain:  
Daily requirement: 150  $\mu\text{g}$ ; The golden rice: 31  $\mu\text{g/g}$

爭議：1. 沒有其他Vit A來源？2. GMO的特洛伊木馬？3. 環境等未知影響

2006/1/31 Dow AgroSciences: the world's first plant-cell-produced vaccine against Newcastle disease virus (禽鳥類)

2012, Pfizer, Elelyso, produce the human enzyme (aliglucerase α) that these Gaucher disease patients lack in **carrot cells**, approved

Advantages of plant over mammalian cells: 1. Cheaper maintenance expense; 2. Less pathogen contamination such as viruses



First transgenic meat!

1. 美國FDA同意不須標示為基改食品。Why?台灣？
2. 封閉環境飼養
3. 3倍體、無繁殖能力
4. 16-18 vs. 36月飼養
5. 2017開始供應

## 12.9 GMOs raise questions and concerns

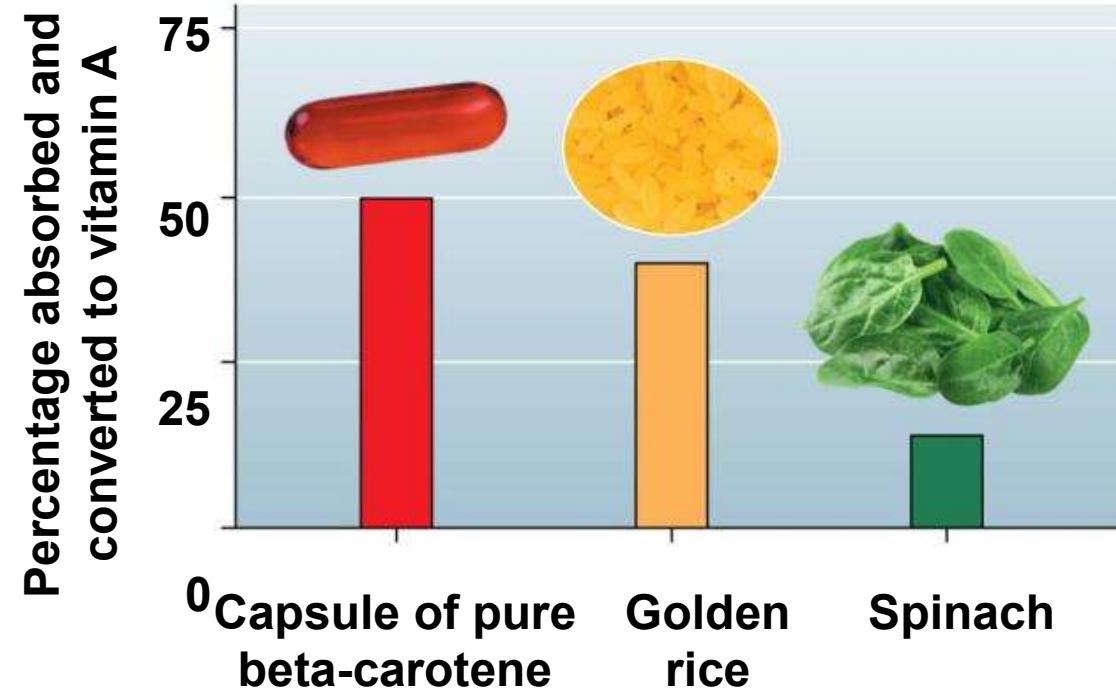
- Scientists use **safety measures** to guard against production and release of new pathogens.
- To guard against rogue microbes, scientists developed a set of guidelines, including
  - **strict laboratory safety** and containment procedures,
  - the genetic crippling of transgenic organisms to ensure that they cannot survive outside the laboratory, and
  - a prohibition on certain obviously dangerous experiments.
- Today, most public concern centers on whether GMOs are safe for humans and the environment.
- Genetically modified organisms are used in crop production because they are more nutritious or cheaper to produce.

## 12.9 Genetically modified organisms raise health concerns

- But do these advantages come at a cost to the health of people consuming GMOs?
- A 2012 animal study involved 104 pigs that were divided into two groups.
  - One group was fed a diet containing 39% GMO corn.
  - Another group was fed a closely related non-GMO corn.
- The health of the pigs—in terms of growth, organ structure, and immune response against foreign DNA—was measured in the short term (31 days), in the medium term (110 days), and over the normal generational life span.
- The researchers reported **no significant differences** between the two groups and no traces of foreign DNA in the slaughtered pig.

- Although pigs are a good model organism for human digestion, critics argue that human data are required to draw conclusions about the safety of dietary GMOs in people.

- A human study of Golden Rice



- was conducted jointly by Chinese and American scientists and published in 2012 and
- concluded that GMO rice can indeed be effective in preventing vitamin A deficiency among children who rely on rice as a staple food.

- To date, no study has documented health risks in humans from GMO foods and there is general agreement among scientists that the GMO foods on the market are safe.
- On the other hand, because they are new, it is not yet possible to measure the **long-term effects** (if any) of GMOs on human health.
- Concerns remain that transgenic plants might
  - **pass** their new genes to related species in nearby wild areas and
  - **disturb** the composition of the natural ecosystem.

- Although the majority of several staple crops grown in the United States—including corn and soybeans—are genetically modified, products made from GMOs are not required to be labeled in any way.
  - In 2012, citizens of California **voted down** (53% to 47%) a ballot measure requiring GMO labeling of food and drink in that state.
  - Why? price, law suits, bureaucracy ...; BUT non-GMO label increase!
  - 那些廠商反對？可口可樂、百事可樂、通用磨坊、卡夫、家樂氏、康尼格拉（使用基因改造玉米、黃豆與甜菜）。研發與銷售基因改造種子和農藥的企業，如孟山都、杜邦先鋒、陶氏農業生技公司（Dow AgroSciences）

- In the case of GMO crops, **zero risk** is probably unattainable.
- Scientists and the public need to weigh the possible benefits versus risks on a case-by-case basis.
- The best scenario would be to proceed with caution, basing our decisions on sound scientific information rather than on either irrational fear or blind optimism.



Roundup Ready® Xtend Crop System for soybeans is intended to provide farmers with more consistent, flexible control of weeds, especially tough-to-manage and glyphosate-resistant weeds, and to help maximize crop yield potential.

WHO: Frequently asked questions on genetically modified foods

[http://www.who.int/foodsafety/areas\\_work/food-technology/faq-genetically-modified-food/en/](http://www.who.int/foodsafety/areas_work/food-technology/faq-genetically-modified-food/en/)

How Monsanto And Scofflaw Farmers Hurt Soybeans In Arkansas

<https://www.npr.org/sections/thesalt/2016/08/01/487809643/crime-in-the-fields-how-monsanto-and-scofflaw-farmers-hurt-soybeans-in-arkansas>

"Xtend," isn't just engineered to tolerate sprays of glyphosate, aka Roundup. It's also immune to dicamba, an illegal herbicide. Dicamba causes damages to general soybeans.



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# 公告資訊

## 我國基因改造食品查驗登記、標示等管理之說明 【發布日期：2014-04-18】

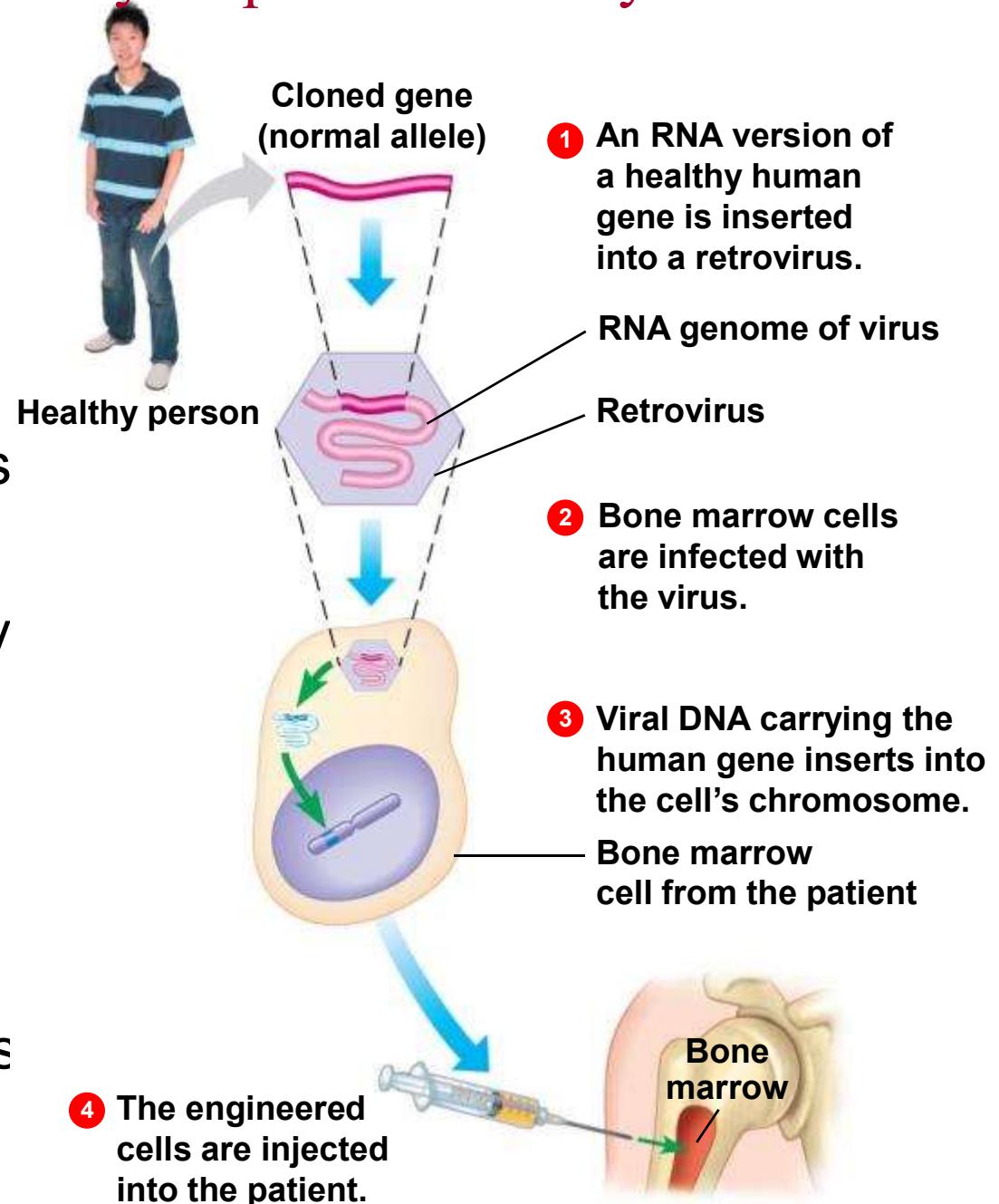
國內對於基因改造食品，係依據食品安全衛生管理法嚴格管理，除須符合各項食品衛生安全規定外，亦有基因改造食品原料之安全性評估、查驗登記及標示等管理規定。為強化基因改造食品標示之管理，食品藥物管理署預計於四月底召開專家會議，並研擬修正有關基因改造食品標示規定。

國際間基因改造蓄豆及玉米，從研發到商業化過程，必須經過嚴謹之食品安全評估，並我國則由科技部、農委會和衛福部分別在上、中、下游，就所管實驗室研究、田間試驗和食品衛生等方面，做安全評估的層層把關。

**無子西瓜**：用秋水仙素（colchicine）處理普通西瓜的種子或子苗的生長點，可以使體細胞的染色體加倍，成為4倍體。再用4倍體西瓜作為母本，2倍體西瓜作父本，雜交後可得3倍體。以3倍體種子培育出來的西瓜，染色體不能進行正常分裂，染色體組不完整，基因間失去平衡作用，缺乏繁殖能力，種子無法正常受精發育而變成白色細嫩的秕（ㄉ一），在學術上稱為3倍體西瓜，也就是俗稱的「無子西瓜」。

## 12.10 Gene therapy may someday help treat a variety of diseases

- **Gene therapy** is the alteration of a diseased individual's genes for therapeutic purposes.  
Functional allele
- One possible procedure is the following:
  1. A gene from a healthy person is cloned, converted to an RNA version, and then inserted into the RNA genome of a harmless virus.



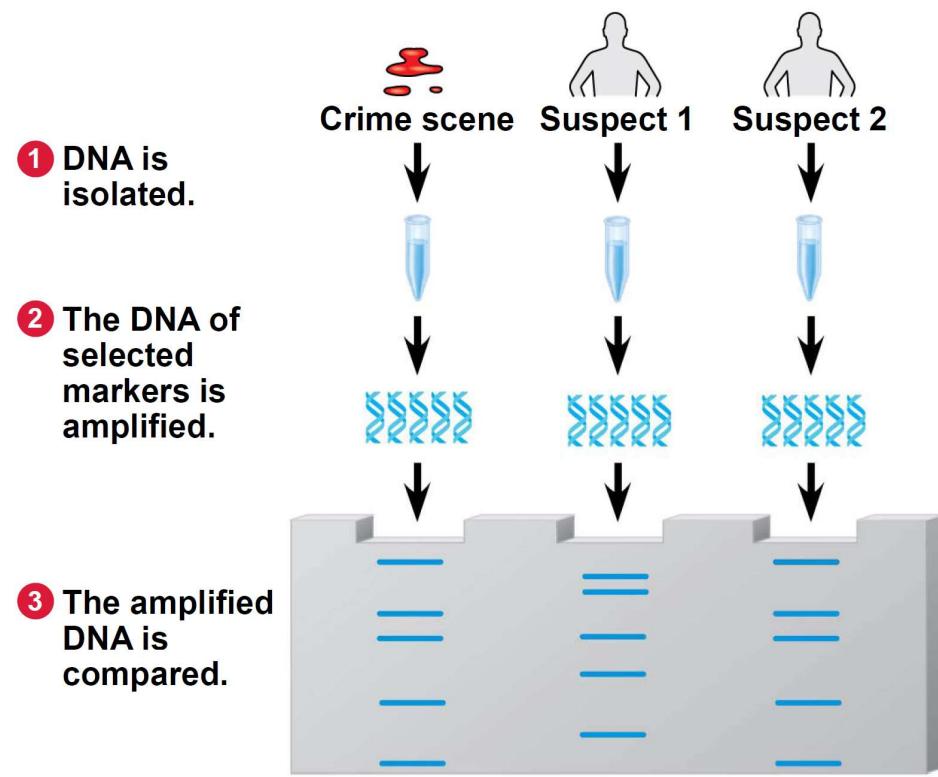
2. Bone marrow cells are taken from the patient and infected with the recombinant virus.
  3. The virus inserts a DNA version of its genome, including the normal human gene, into the cells' DNA.
  4. The engineered cells are then injected back into the patient.
- The promise of gene therapy thus far exceeds actual results, but there have been some successes in the treatment of severe combined immunodeficiency (SCID) and Leber's congenital amaurosis (LCA).
  - The first successful human gene therapy trial in 2000
    - helped nine SCID patients, but
    - **caused leukemia** in three of the patients, and
    - resulted in one death.

- The use of gene therapy raises many questions.
  - Some critics suggest that tampering with human genes in any way will inevitably lead to the practice of eugenics, the deliberate effort to control the genetic makeup of human populations.
  - Other observers see no fundamental difference between the transplantation of genes into somatic cells and the transplantation of organs.
  - How can we build in **gene control** mechanisms that make appropriate amounts of the product at the right time and place?
  - How can gene insertion be performed **without harming** other cell functions?
  - Should we try **to eliminate genetic defects** in our children and descendants when genetic variety is a necessary ingredient for the survival of a species?

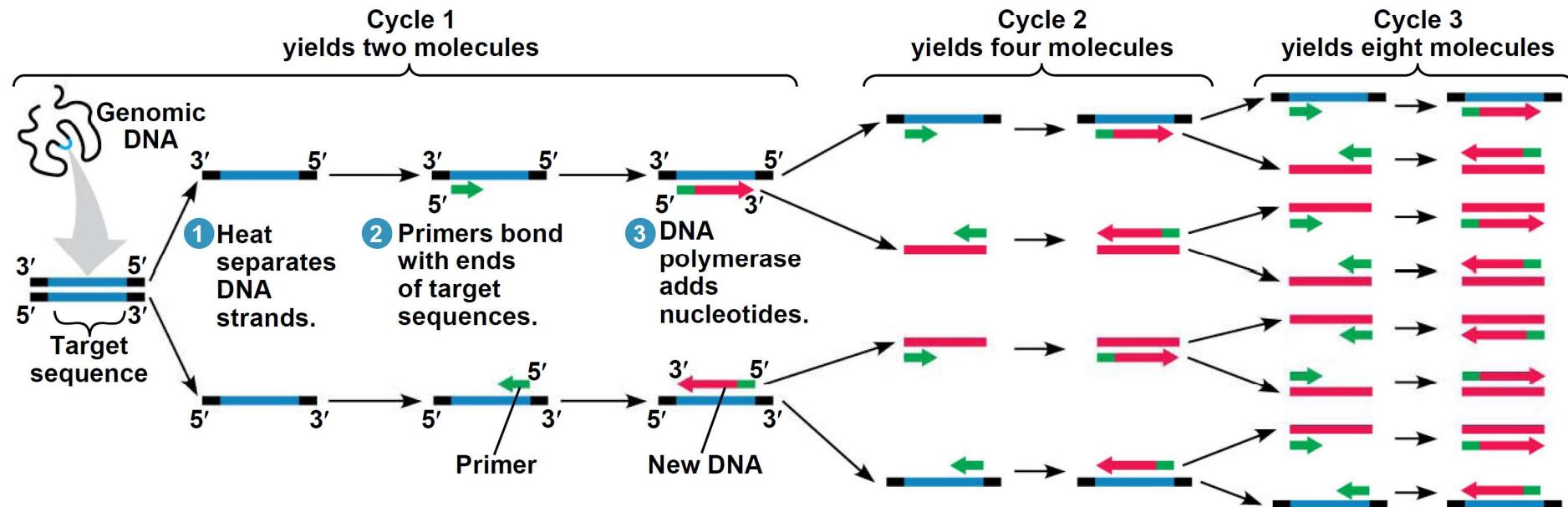
# DNA PROFILING

## 12.11 The analysis of genetic markers can produce a DNA profile

- **DNA profiling** is the analysis of DNA samples to determine whether they come from the same individual.
- In a typical investigation involving a DNA profile:
  1. DNA samples are isolated from the crime scene, suspects, victims, or other evidence
  2. selected markers from each DNA sample are amplified (copied many times), producing a large sample of DNA fragments
  3. the amplified DNA markers are compared, providing data about which samples are from the same individual.



## 12.12 The PCR method is used to amplify DNA sequences



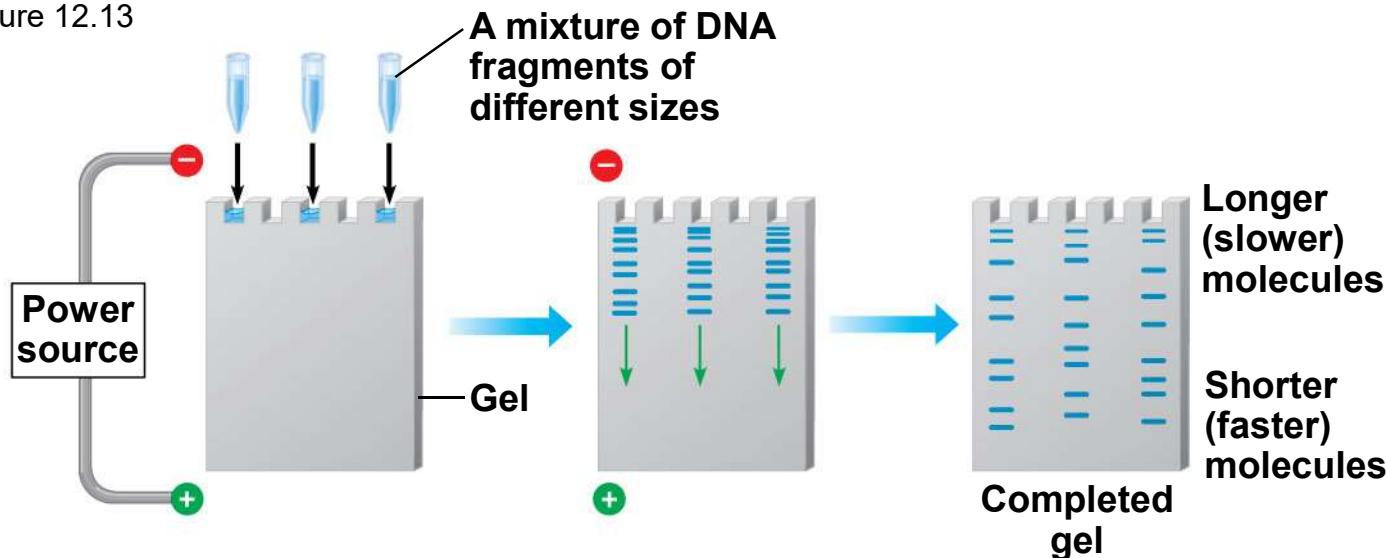
- **Polymerase chain reaction (PCR)** is a technique by which a specific segment of a DNA molecule can be targeted and quickly amplified in the laboratory. 聚合酶鏈鎖反應
- PCR relies upon a pair of short **primers**, which are chemically synthesized, single-stranded DNA molecules with sequences that are complementary to sequences at each end of the target sequence.

- One primer is complementary to one strand at one end of the target sequence.
  - The second primer is complementary to the other strand at the other end of the sequence.
  - The primers thus bind to sequences that flank the target sequence, marking the start and end points for the segment of DNA being amplified.
- The basic steps of PCR are as follows:
1. The reaction mixture is heated to separate the strands of the DNA double helices.
  2. The strands are cooled. As they cool, primer molecules hydrogen-bond to their target sequences on the DNA.
  3. A heat-stable DNA polymerase builds new DNA strands by extending the primers in the  $5' \rightarrow 3'$  direction.
- These three steps are repeated over and over, doubling!

- The advantages of PCR include
  - the ability to **amplify** DNA from a small sample,
  - obtaining results **rapidly**, and
  - a reaction that is highly **sensitive**, copying only the target sequence.
- Devised in 1985, PCR has had a major impact on biological research and biotechnology. PCR has been used to amplify DNA from
  - fragments of ancient DNA from a mummified human,
  - a 40,000-year-old frozen woolly mammoth,
  - a 30-million-year-old plant fossil, and
  - DNA from fingerprints or from tiny amounts of blood, tissue, or semen found at crime scenes.

- Many DNA technology applications rely on **gel electrophoresis**, a method that separates macromolecules, usually proteins or nucleic acids, on the basis of size, electrical charge, or other physical properties.
- **Gel electrophoresis** can be used to separate DNA molecules based on **size** as follows:
  1. A DNA sample is placed at one end of a porous gel.
  2. Current is applied and DNA molecules move from the negative electrode toward the positive electrode.
  3. Shorter DNA fragments move through the **gel matrix** more quickly and travel farther through the gel.
  4. DNA fragments appear as bands, visualized through staining or detecting radioactivity or fluorescence.
  5. Each band is a collection of DNA molecules of the same length.

Figure 12.13



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Backbone  $\text{PO}_4^-$   
Video: Biotechnology Lab



## The Nobel Prize in Chemistry 1993



Kary B. Mullis  
Prize share: 1/2



Michael Smith  
Prize share: 1/2

專利？

The Nobel Prize in Chemistry 1993 was awarded "for contributions to the developments of methods within DNA-based chemistry" jointly with one half to Kary B. Mullis "for his invention of the polymerase chain reaction (PCR) method" and with one half to Michael Smith "for his fundamental contributions to the establishment of oligonucleotide-based, site-directed mutagenesis and its development for protein studies".

## 12.14 STR analysis is commonly used for DNA profiling

- **Repetitive DNA** consists of nucleotide sequences that are present in multiple copies in the genome.
- **Short tandem repeats (STRs)** are short nucleotide sequences that are repeated in tandem, 串聯
  - composed of **different numbers of repeating units** in individuals and
  - used in DNA profiling.
- **STR analysis**
  - compares the lengths of STR sequences at specific sites in the genome and
  - typically analyzes 13 different STR sites.
  - 4 nucleotide repeats

FBI CODIS: combined DNA Index System; start 1990, 13 STR sites, 1/10 billion (100億); increase to 20, 2017/1/1

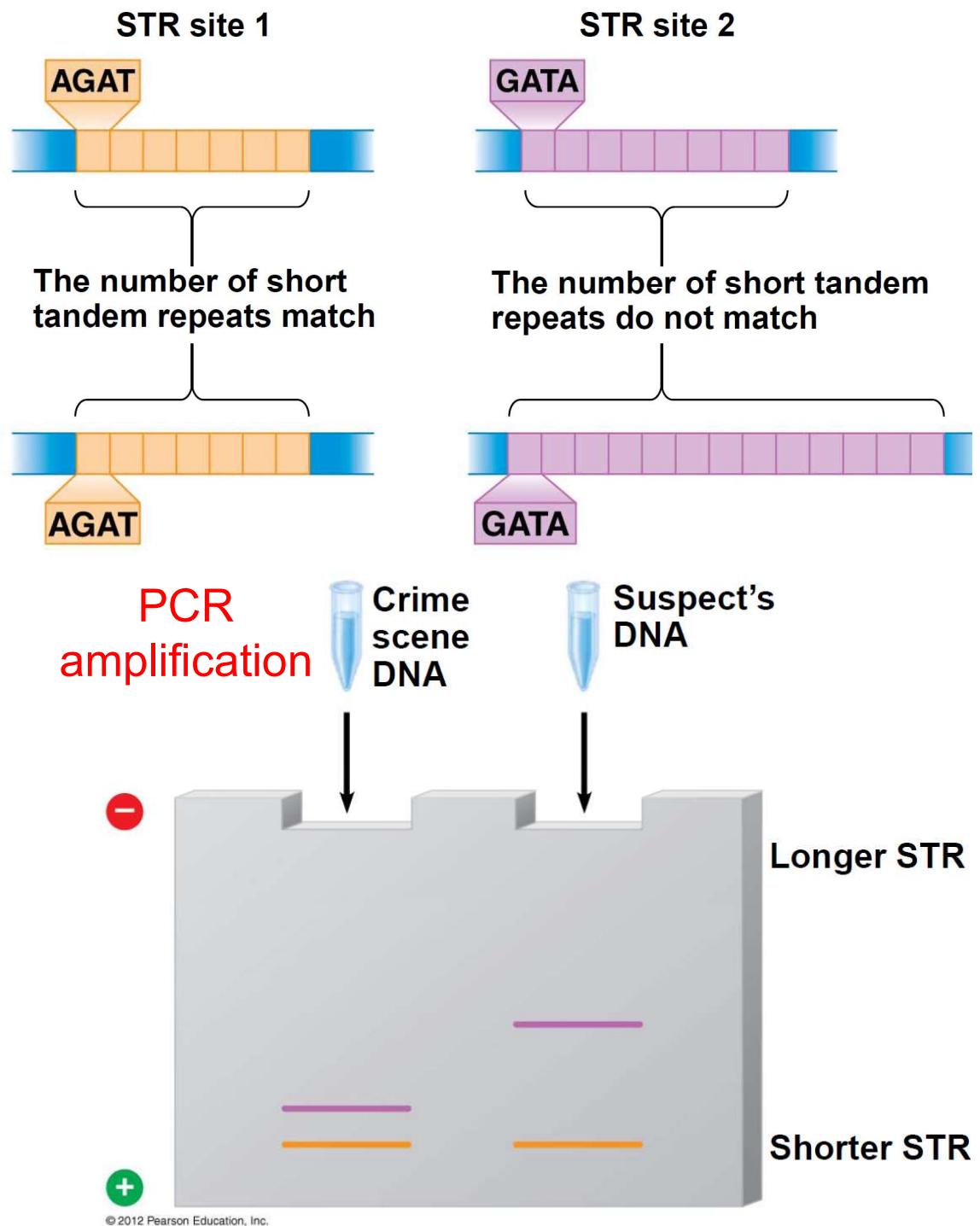
## 刑事警察局DNA鑑定

<https://www.cib.gov.tw/Science/DNA>

<http://www.idealversion.com/biomedicine/archives/010v3n1/bm-09-24.pdf>

表四：DNA STR分析法運用項目（摘錄部份）<sup>33</sup>。

運用項目	比對方式
D N A 鑑 定 案 件 ( Forensic DNA Casework )	犯罪現場檢體之DNA與涉嫌人對照檢體（如唾液、血液）DNA直接比對（比對各點位對偶基因之數字是否相同）。
受 刑 人 或 涉 嫌 人 D N A 建 檔 與 比 對 ( Databases for convicted or suspect )	犯罪現場檢體之DNA與檔案之DNA直接比對。
災 難 造 成 大 量 死 亡 案 件 ( Mass fatalities from disasters )	家屬DNA與遺體DNA之血緣關係比對。
失 蹤 人 口 比 對 ( Missing Persons )	家屬DNA與所有已發現之遺體DNA之血緣關係比對、新發現遺體與所有家屬DNA檔案比對。
血 緣 關 係 鑑 定 ( Human relationship testing )	應受測者要求進行一親等（父母子女）、二親等（手足、半手足）或其他更遠親等（叔侄）之比對。
骨 髓 移 植 監 控 ( Monitoring of bone marrow engrafting )	觀察被移植者血液是否有移植者之DNA，或完全被取代。



## 12.15 DNA profiling has provided evidence in many forensic investigations

- DNA profiling is used to
  - determine guilt or innocence in a crime,
  - settle questions of paternity, and
  - probe the origin of nonhuman materials.



Released 2001.2.12; currently is married and lives in Virginia Beach.  
He was awarded a \$2.25M settlement

**TABLE 12.15 STR Analysis Data that Exonerated Earl Washington**

Source of Sample	STR Marker 1	STR Marker 2	STR Marker 3
Semen on victim	17, 19	13, 16	12, 12
Earl Washington	16, 18	14, 16	11, 12
Kenneth Tinsley	Guilty! 17, 19	13, 16	12, 12

- Lawyers at the Innocence Project, a nonprofit organization dedicated to overturning wrongful convictions, used DNA technology and legal work to exonerate more than 300 convicted criminals since 1989, including 17 who were on death row.
- 911 World Trade Center: >20,000 victims' remains
  - >50% confirmed by DNA evidence only
- 2004 Tsunami victims
- 2011, Osama bin Laden: compared with his relatives
- 2014 澎湖空難，DNA人別鑑定
- Paternity of historical interest

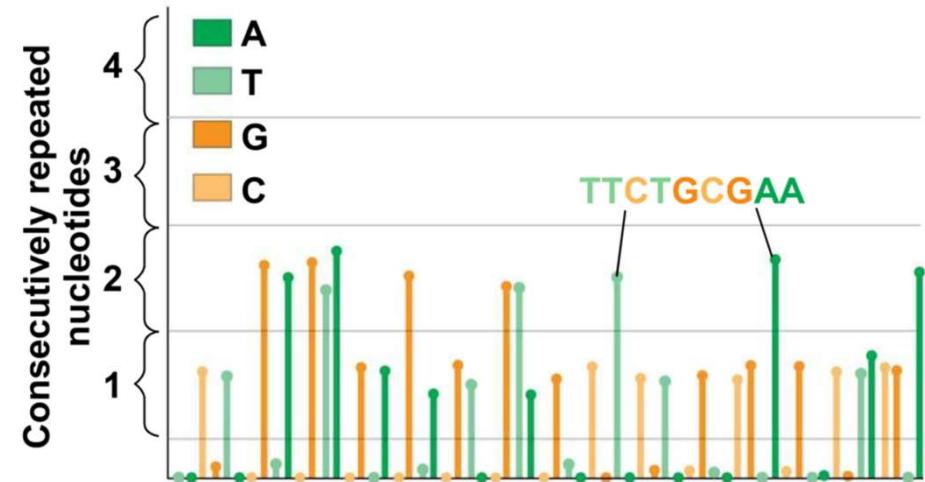
**GENOMICS**

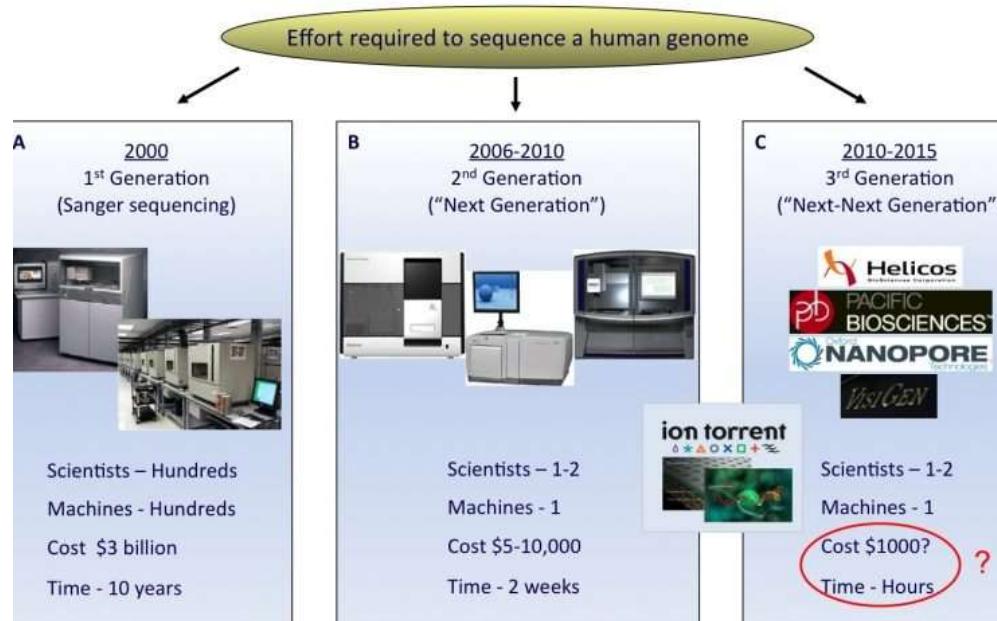
And

**Bioinformatics**

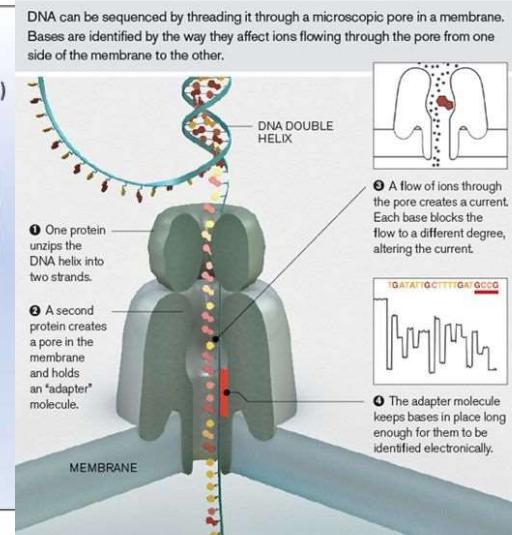
## 12.16 Small segments of DNA can be sequenced directly

- Next- and third-generation sequencing machines can quickly determine the sequence of relatively short stretches of DNA.
- 2001, next-generation sequencing (NGS): monitor the nucleotide added during synthesis from a short DNA template
- The process can be rapid and in-expensive (but the machine ...)
- Third-generation: single, very long DNA; by moving it along a nanopore; 2015 marketing





## 3<sup>rd</sup> Gen. Sequencing: Oxford Nanopore

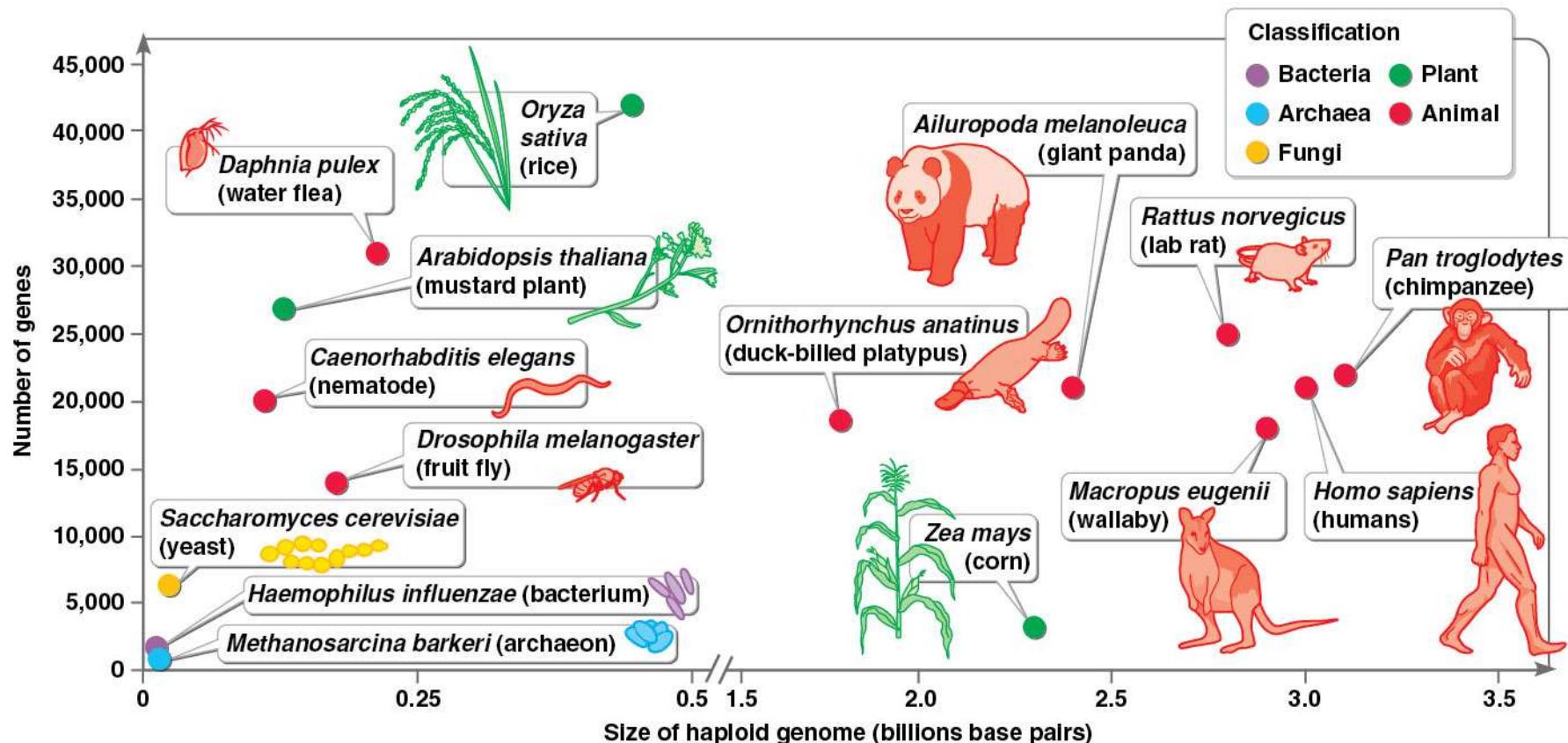


<https://www.europeanpharmaceuticalreview.com/article/10409/dna-sequencing-technologies-and-emerging-applications-in-drug-discovery/>

<https://slideplayer.com/slide/7899792/>

## 12.17 Genomics is the scientific study of whole genomes

- **Genomics** is the study of complete sets of genes.
- Genomics researchers have sequenced many prokaryotic and eukaryotic genomes.
- Besides being of interest in their own right, nonhuman genomes can be compared with the human genome.



- Genomics allows another way to examine evolutionary relationships.
  - Genomic studies showed a 96% similarity in DNA sequences between chimpanzees and humans.
  - Functions of human disease-causing genes have been determined by comparing human genes to similar genes in yeast.

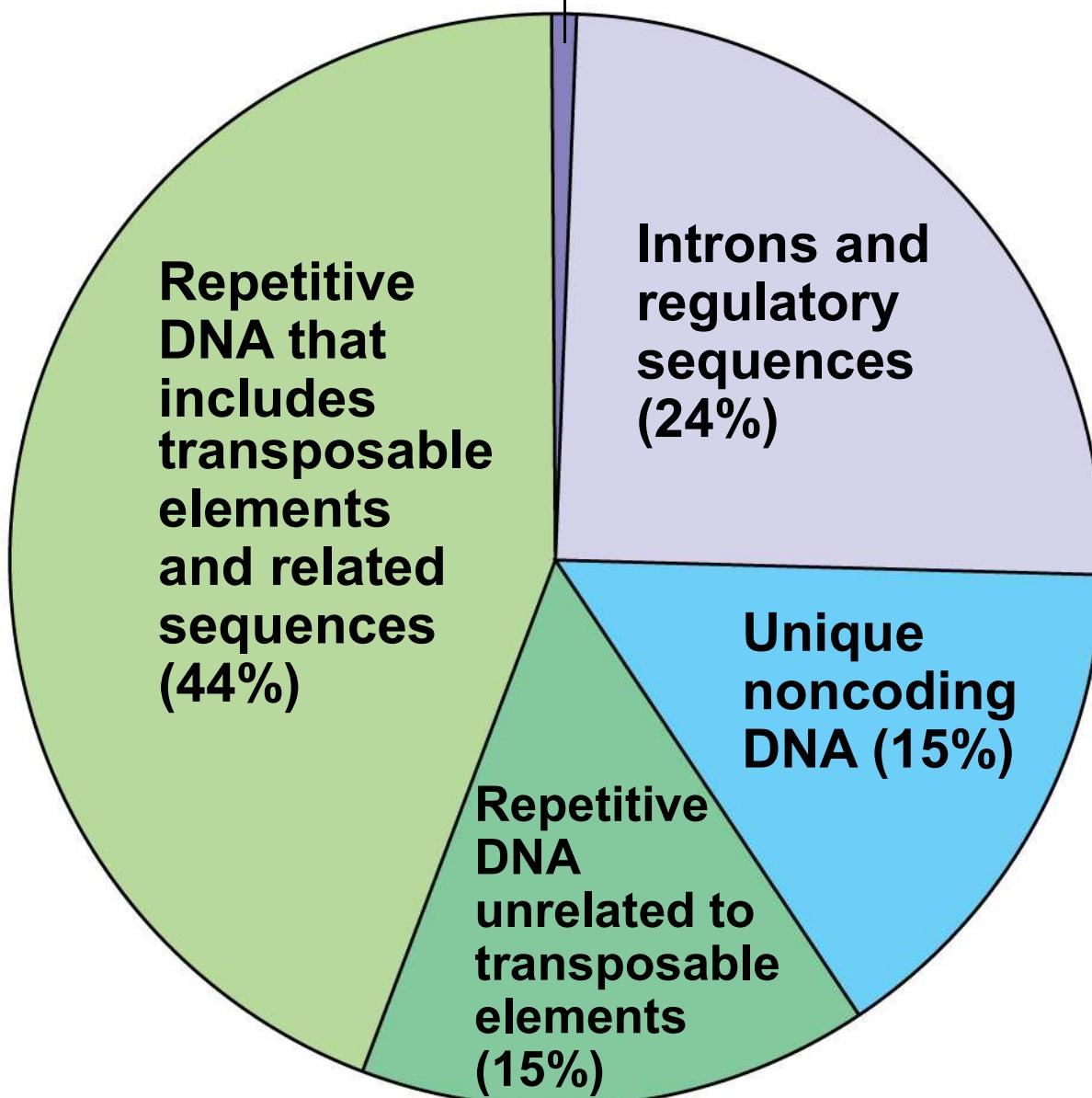
## 12.18 The Human Genome Project revealed that most of the human genome does not consist of genes

- The goals of the **Human Genome Project (HGP)** included 1990; Taiwan Chromosome 4
  - determining the nucleotide sequence of all DNA in the human genome and
  - identifying the location and sequence of every human gene.
- Results of the Human Genome Project indicate that
  - humans have about 21,000 genes in 3 billion (32億) nucleotide pairs,
  - only 1.5% of the DNA codes for proteins, tRNAs, or rRNAs, and
  - the remaining 98.5% of the DNA is noncoding DNA including

- Much of the DNA between genes consists of repetitive DNA, nucleotide sequences present in many copies in the genome.
- Stretches of DNA with thousands of short repetitions are also prominent at
  - the centromeres and
  - ends of chromosomes—called **telomeres**.
- In a second main type of repetitive DNA, each repeated unit is hundreds of nucleotides long, and the copies are scattered around the genome.
- Most of these sequences seem to be associated with **transposable elements** (“jumping genes”), DNA segments that can move or be copied from one location to another in a chromosome and even between chromosomes.

Figure 12.18

### Exons (regions of genes coding for protein or giving rise to rRNA or tRNA) (1.5%)

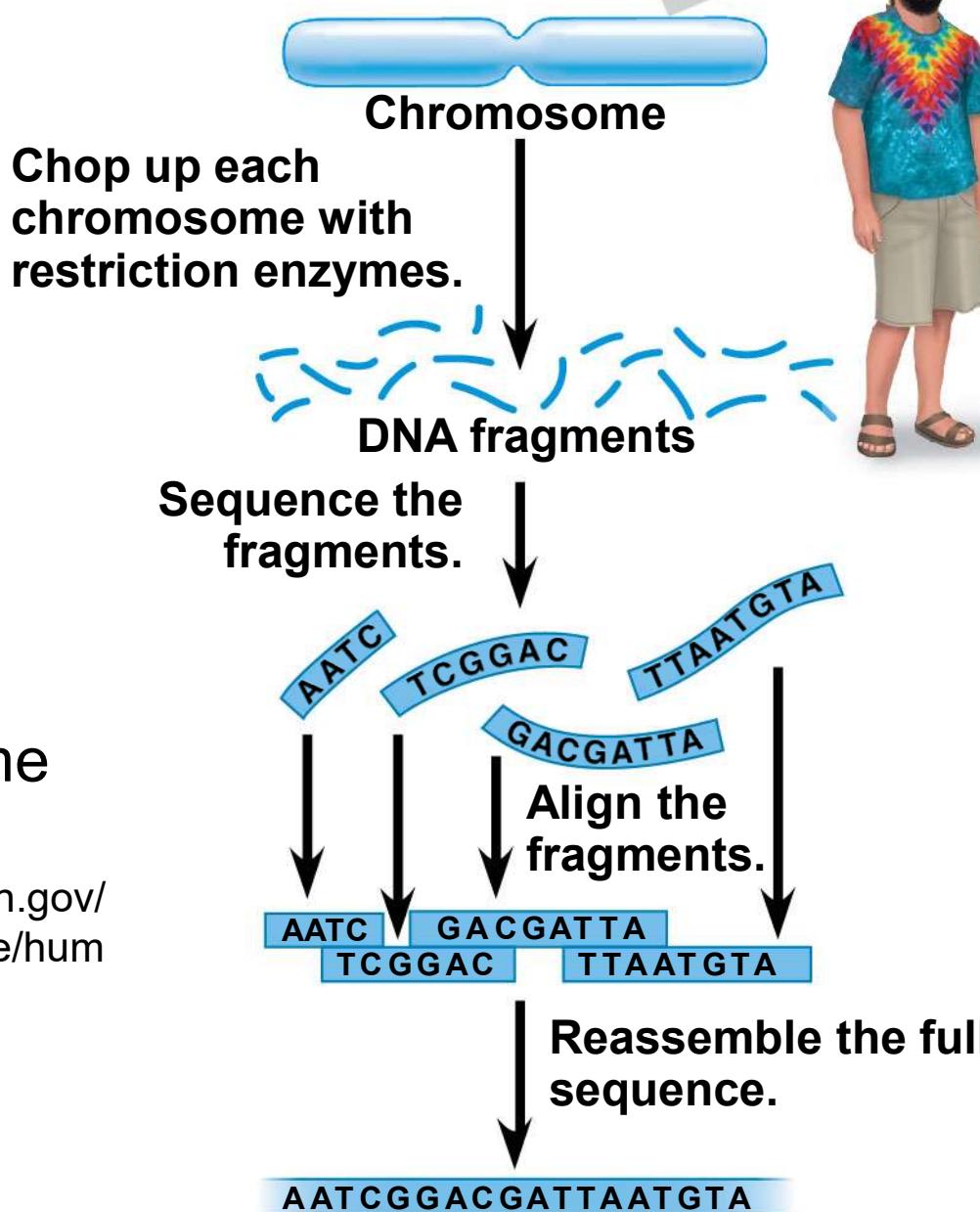


12.19 The whole-genome shotgun method of sequencing a genome can provide a wealth of data quickly

- The Human Genome Project proceeded through **three stages** that provided progressively more detailed views of the human genome.  
精準但慢
  1. A low-resolution *linkage map* was developed using **RFLP analysis** of 5,000 genetic markers.
  2. A **physical map** was constructed from nucleotide distances between the linkage-map markers.
  3. DNA sequences for the mapped fragments were determined.

- The **whole-genome shotgun method**
  - was proposed in 1992 by molecular biologist J. Craig Venter,
  - He used restriction enzymes to produce fragments that were cloned and sequenced in just one stage and ran high-performance computer analyses to assemble the sequence by aligning overlapping regions.
- Today, this whole-genome shotgun approach is the method of choice for genomic researchers because it is
  - relatively fast and
  - inexpensive.
- However, limitations of the whole-genome shotgun method suggest that a **hybrid approach** using genome shotgunning and physical maps may prove to be the most useful.

# whole-genome shotgun method

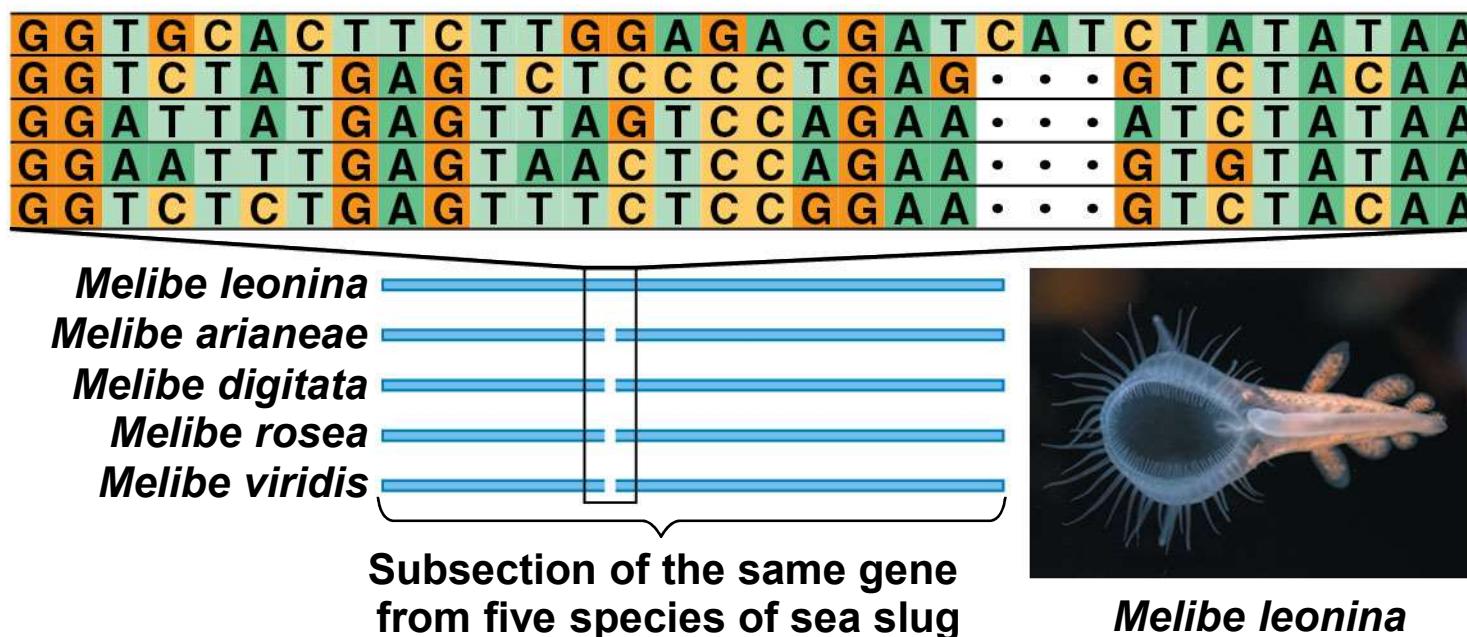


## Human Genome Resources

<http://www.ncbi.nlm.nih.gov/projects/genome/guide/human/index.shtml>

## 12.20 The field of bioinformatics is expanding our understanding of genomes

- **Bioinformatics**, the use of computational methods to analyze biological data, can be used to analyze large sets of data about DNA sequences and proteins.
- NCBI database: <https://www.ncbi.nlm.nih.gov/>
- **Proteomics** involves similar systematic studies of the full protein sets (proteomes) encoded by genomes.



## ■ Proteomics 蛋白質體學

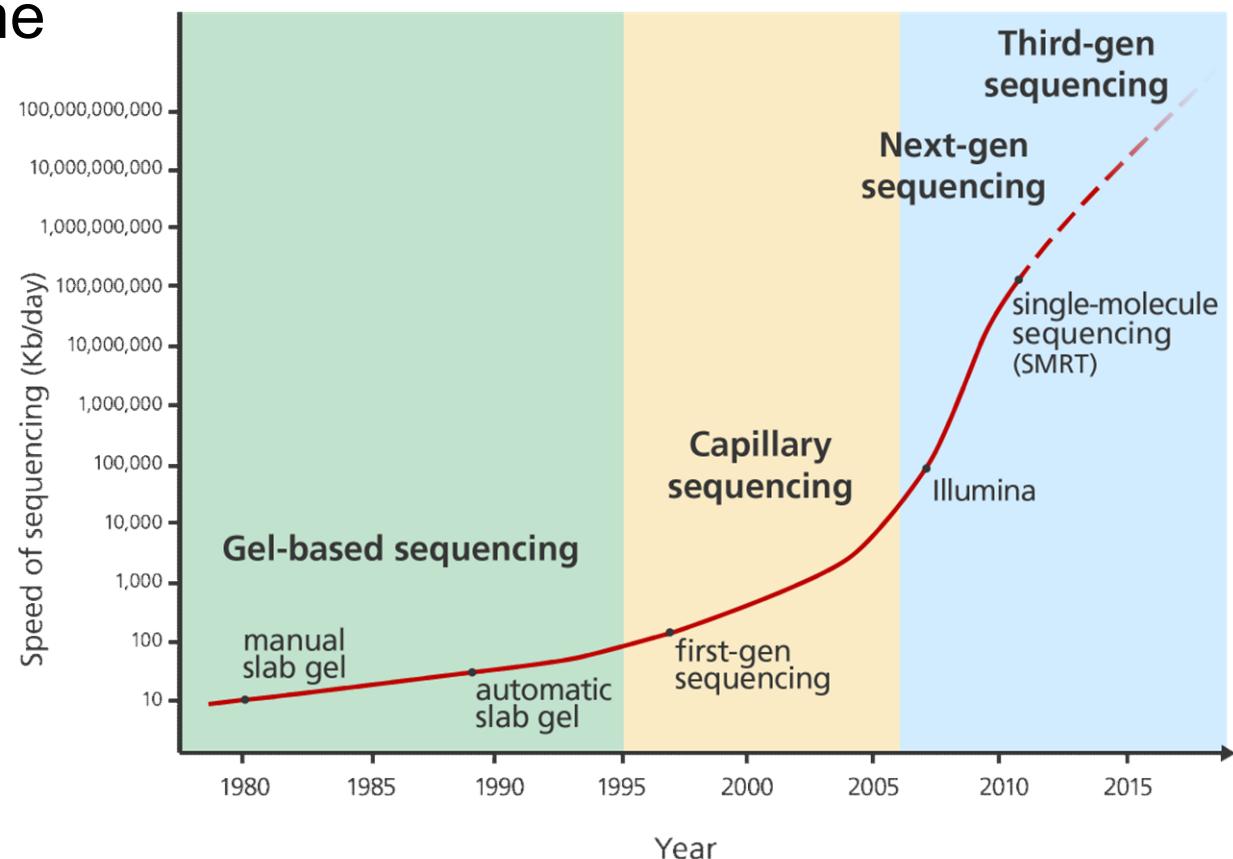
- is the study of the full protein sets encoded by genomes and
- investigates protein functions and interactions.

■ The human proteome includes about 100,000 proteins.

■ Genomics and proteomics are helping biologists study life from an increasingly holistic approach.

## Metabolomics 代謝體學

Big Data

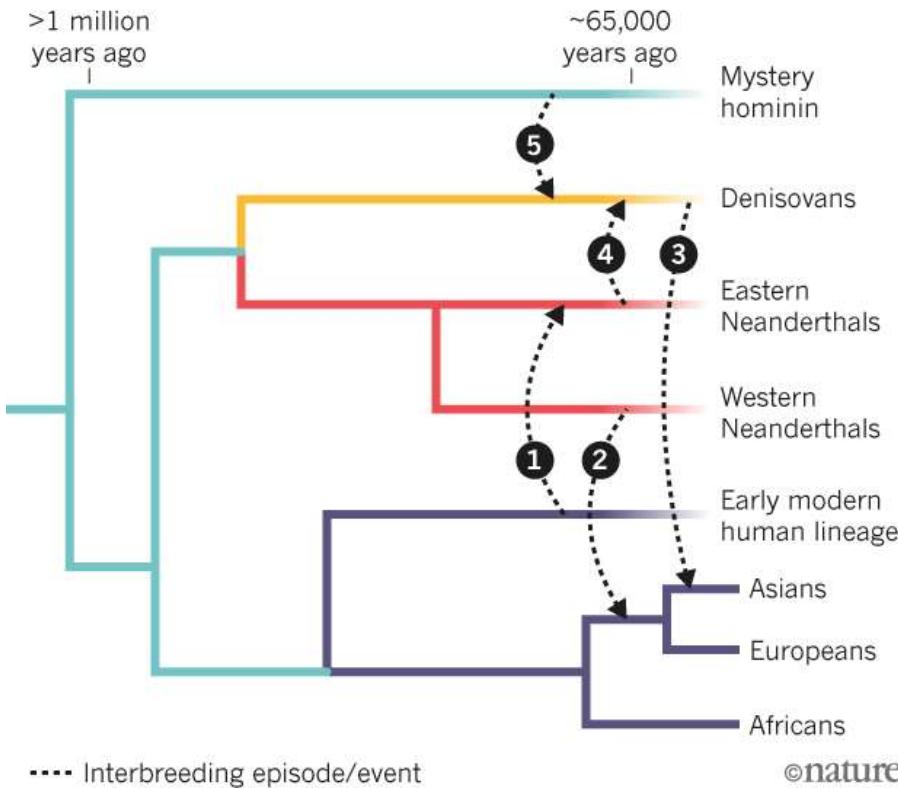


## 12.21 Genomes hold clues to human evolution

- Human and chimp genomes differ by
  - 1.2% in single-base substitutions and
  - 2.7% in insertions and deletions of larger DNA sequences.
- Genes showing rapid **evolution** in humans include
  - genes for defense against malaria and tuberculosis,
  - a gene regulating brain size, and 結核病
  - the ***FOXP2*** gene involved with speech and vocalization.
- Neanderthals: 2013 sequenced
  - close human relatives (closer than chimpanzee)
  - extinct 30,000 yrs ago (現代人出現在歐洲: 50,000 yrs ago)
  - a **separate** species, had the ***FOXP2***
  - Present humans of European and Asian descent obtained Neanderthal-derived genes ~70,000 yrs ago
  - Interbreed!

## A HISTORY OF INTERBREEDING

Early modern humans, Denisovans, and Neanderthals all interbred with each other on multiple occasions in the past 100,000 years.



<https://www.nature.com/news/evidence-mounts-for-interbreeding-bonanza-in-ancient-human-species-1.19394>

1-2% human genome were from Neanderthals

Neanderthals were lactose intolerant

Keratin production: influence the hair, nail, and skin

## You should now be able to

1. Explain how plasmids are used in gene cloning.
2. Explain how restriction enzymes are used to “cut and paste” DNA into plasmids.
3. Explain how plasmids, phages, and BACs are used to construct genomic libraries.
4. Explain how a cDNA library is constructed and how it is different from genomic libraries constructed using plasmids or phages.
5. Explain how a nucleic acid probe can be used to identify a specific gene.
6. Explain how different organisms are used to mass-produce proteins of human interest.
7. Explain how DNA technology has helped to produce insulin, growth hormone, and vaccines.
8. Explain how genetically modified (GM) organisms are transforming agriculture.
9. Describe the risks posed by the creation and culturing of GM organisms and the safeguards that have been developed to minimize these risks.

10. Describe the benefits and risks of gene therapy in humans. Discuss the ethical issues that these techniques present.
11. Describe the basic steps of DNA profiling.
12. Explain how PCR is used to amplify DNA sequences.
13. Explain how gel electrophoresis is used to sort DNA and proteins.
14. Explain how short tandem repeats are used in DNA profiling.
15. Describe the diverse applications of DNA profiling.
16. Explain how restriction fragment analysis is used to detect differences in DNA sequences.
17. Explain why it is important to sequence the genomes of humans and other organisms.
18. Describe the structure and possible functions of the noncoding sections of the human genome.
19. Explain how the human genome was mapped.
20. Compare the fields of genomics and proteomics.
21. Describe the significance of genomics to the study of evolutionary relationships and our understanding of the special characteristics of humans.