Biotechnology

HGP: intro
http://www.youtube.com/watch?v=XuUpnAz5y1g

Ethical Problems:
http://www.youtube.com/watch?v=gkQJ26DAxfs

Francis Collins, Director of NIH Center for Human Genomics Research:
http://www.youtube.com/watch?v=xP_keLq80eI

Craig Venter, Founder of Celera(57 minutes)
http://www.youtube.com/watch?v=q77Ow4m040E

What is Biotechnology?

- **Definition:** “Techniques that use living organisms to make or modify products, improve plants or animals, and develop microorganisms for specific purposes” (By National Research Council)

- **Characteristics:** Multidisciplinary (cell & molecular biology, microbiology, genetics, physiology, biochemistry, engineering, computer science, etc.)

The Nature of The Gene

- Johann Friedrich Miescher (1868)
  - isolated ‘nuclein’ from the nucli of white blood cells at Univ. Tubingen
- Fred Griffith (1928)
  - Hereditary information had been transferred from the heat-killed pathogenic strain to the harmless strain
- Avery, MacLeod, & McCarty (1944)
  - Hereditary substance in extracts used by Griffith was probably DNA
- Alfred Hershey & Martha Chase (1952)
  - Strong evidence that DNA was the genetic material
- Watson & Crick (1953)
  - Proposed the structural model for DNA
  - Nobel Prize winners (1962)

- 핵산은 스위스의 생물학자 F.Miescher에 의하여 1868년에 처음으로 발견되었다. Miescher는 세포의 핵 속에 들어 있는 물질을 분석하기 위해 적당한 재료를 찾다가 병원에서 붕대에 묻어 나오는 고름을 택하였다. 고름은 백혈구가 파괴된 것이기 때문에 이것을 쓰면 다량의 핵물질을 비교적 용이하게 얻을 수 있는 것이다.
  Miescher는 이 고름에서 핵성분을 분리 추출하여 분석한 결과 강한 산성(酸性)을 나타내며 인(燐)을 함유하는 유기화합물이 들어 있음을 알고, 이 물질에 뉴클레인(nuclein)이라는 이름을 붙였다. 이 뉴클레인은 그 후 핵 속에 들어 있는 산성물질이라는 뜻에서 nucleic acid, 즉 핵산이라 불리게 되었다.

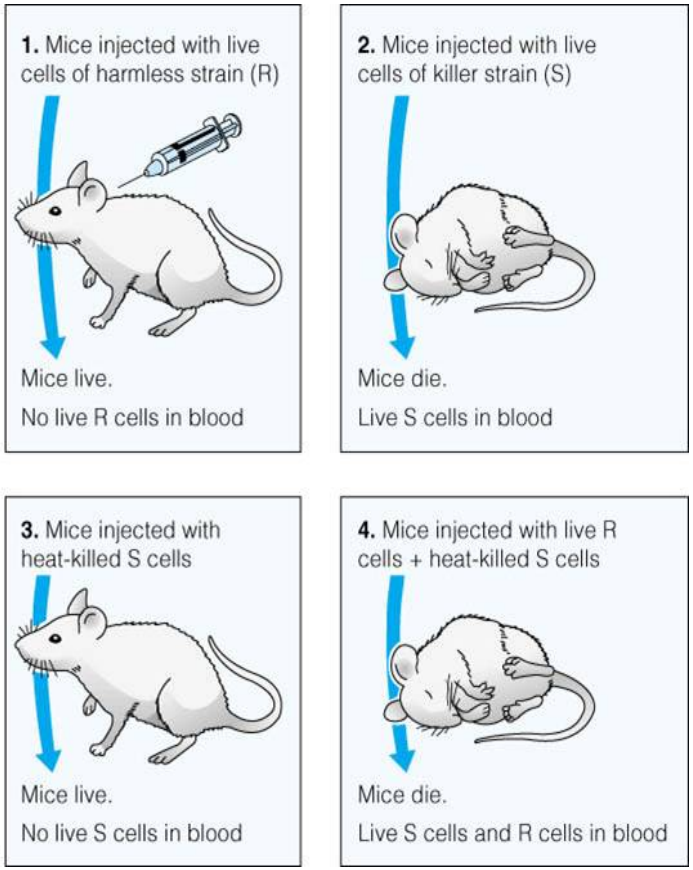
- 1928년 Griffith의 실험에서 폐렴균을 열처리하였더니 쥐에게 폐렴이 발생하지 않는 다는 것을 확인하였고, 무해한 세균과 열처리한 폐렴균을 같이 주사한 쥐는 폐렴으로 사망한 것을 관찰하였다. 그는 열처리된 폐렴균에서 무해한 세균으로 유전적 정보가 이동했다고 결론지었다.
- 1944년 Avery 등은 Griffith 의 실험에서 유전적인 정보를 담고 있던 물질이 DNA 였을 가능성이 높다고 제안하였지만 당시만 해도 DNA 는 유전적인 정보를 가지기에는 너무 간단한 구조라는 의견이 지배적이었다.(뒤에 그림이 자세히 나옴)

- 1952년에 DNA가 유전적인 정보를 담고 있다는 강력한 증거가 제시되었다(뒤에 그림 자세히 나옴)
- 1953년에는 Watson & Crick 에 의해 DNA 의 이중나선 구조가 제시되었고 이들은 Novel Prize를 수상하였다.

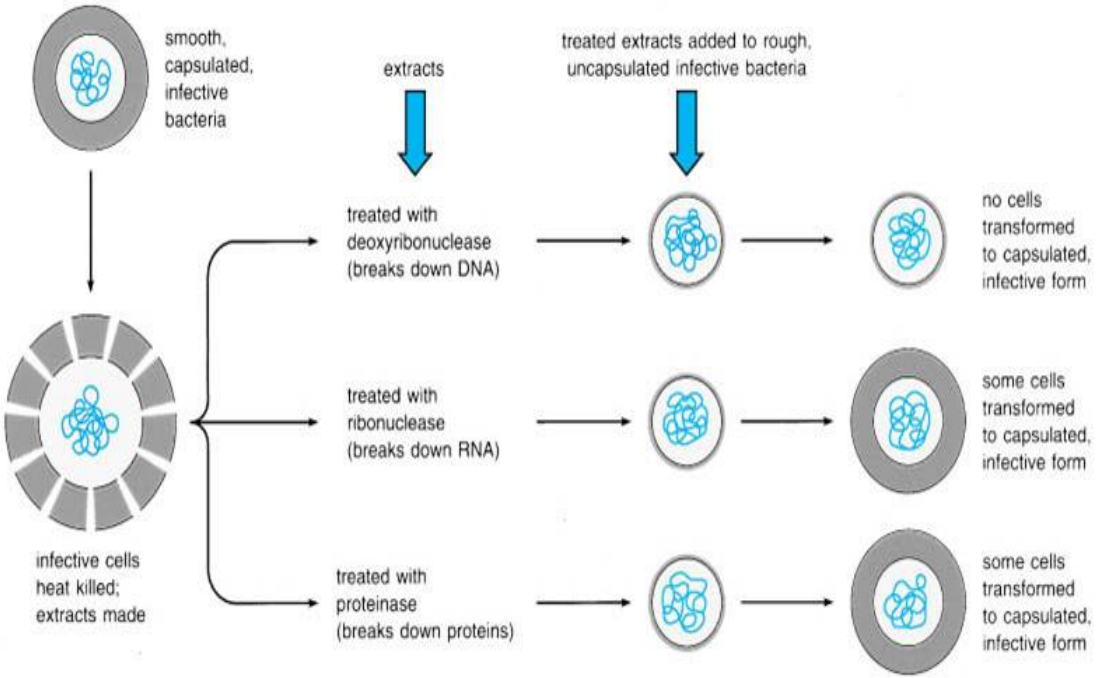
The Nature of The Gene

Transforming principle

1928 - Fred Griffith's Experiment



1944 - Avery, MacLeod, and McCarty expanded Griffith's work



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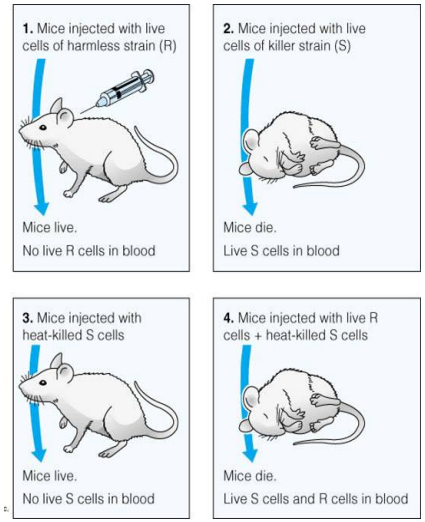
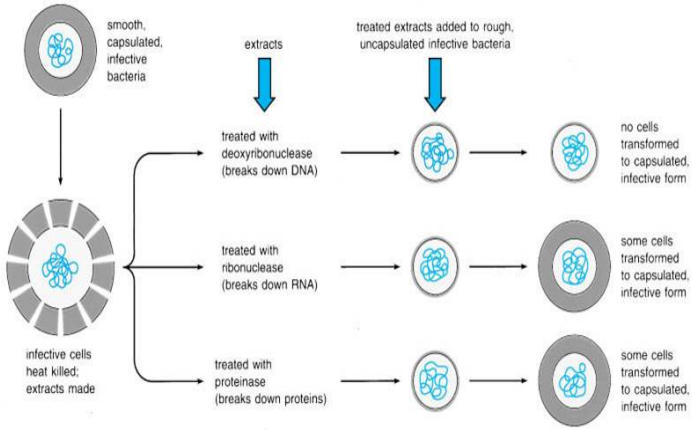
1944년 Avery 등은 세균을 열처리하고 여기에서 추출된 물질을 세 그룹으로 분류하여 실험하였다. 각 그룹에서 추출물을 DNA, RNA, Protein 을 파괴하는 효소에 다시 처리한 후 캡슐이 없는 박테리아에 주입하였더니 DNA사슬이 파괴된 추출물을 넣은 첫 번째 그룹은 캡슐을 형성하지 않았지만, RNA, Protein 이 파괴된 추출물이 주입된 나머지 두 그룹은 캡슐을 형성함을 보고 DNA가 유전적인 정보를 가지고 있다는 이론을 제시하였다.





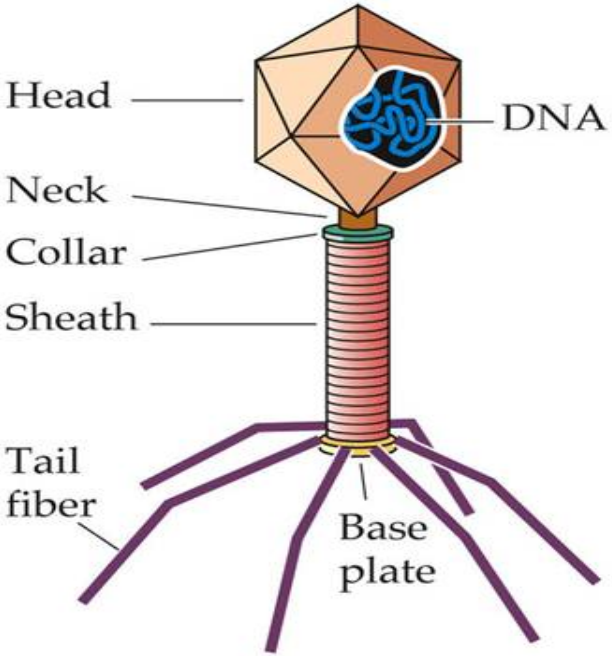
1928 - Fred Griffith's Experiment

1944 - Avery, MacLeod, and McCarty expanded Griffith's work

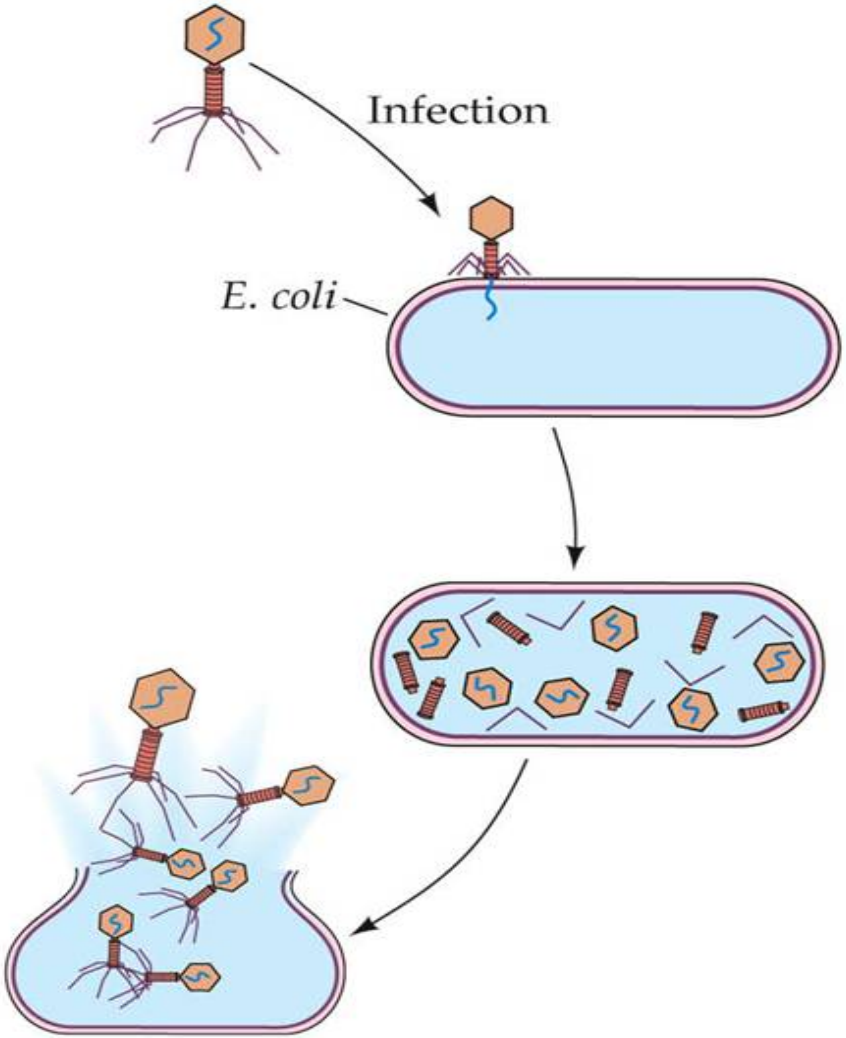
The Nature of The Gene

Alfred Hershey & Martha Chase (1952)

(*a*) **The virus: T2 bacteriophage**



(*b*) **Life cycle of the T2 bacteriophage**

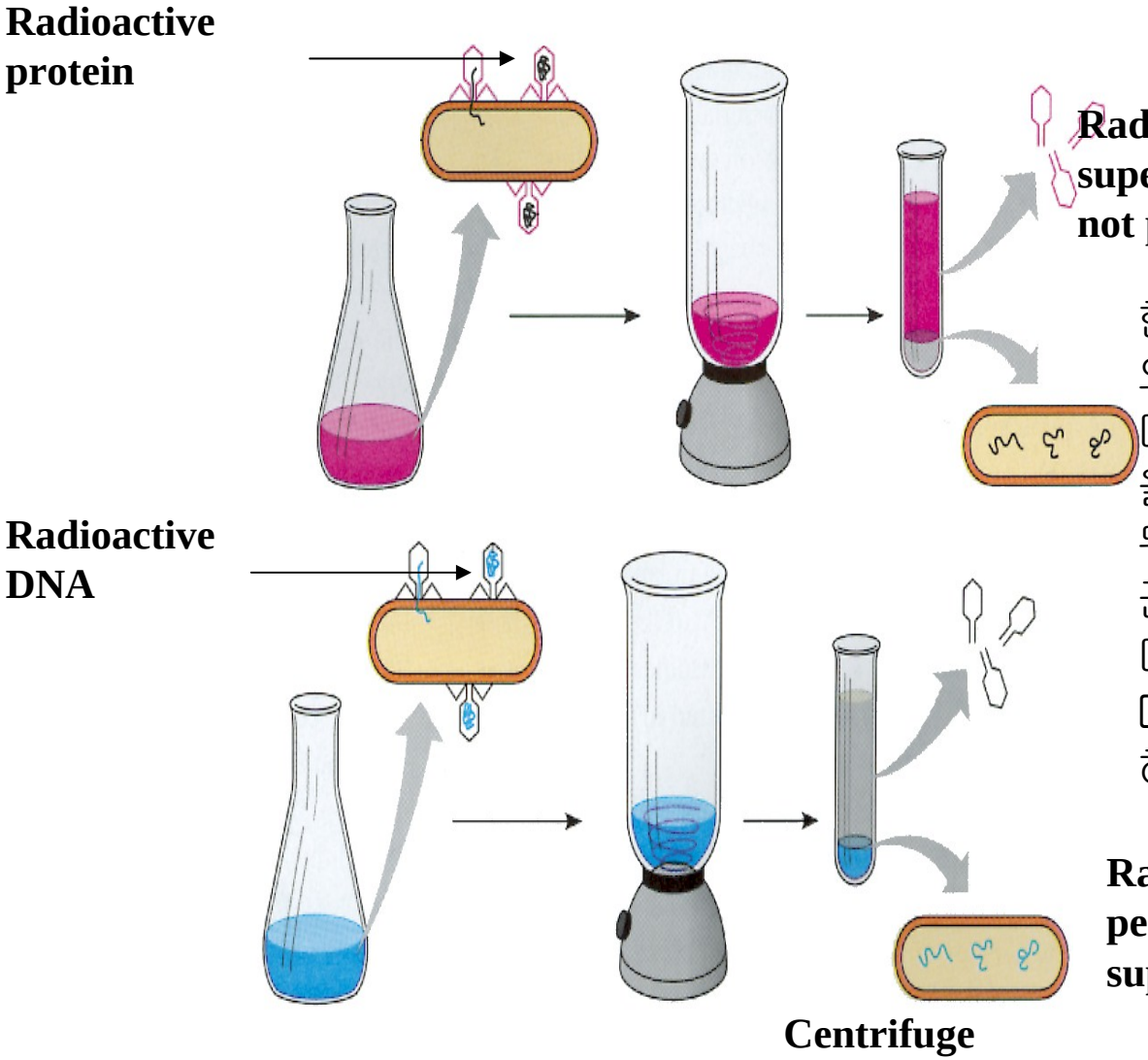


Bacteriophage 는 대장균에 감염을 일으키는 바이러스의 일종이다

E

The Nature of The Gene

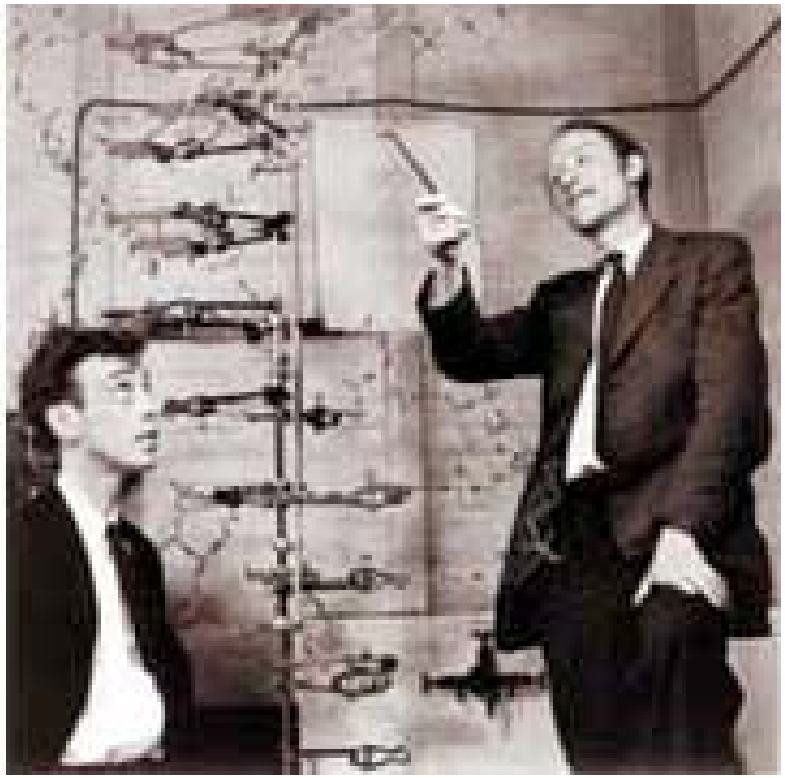
Alfred Hershey & Martha Chase (1952)



한 그룹은 bacteriophage 의 Protein 에 염색을 하고 다른 한 그룹은 DNA에 염색을 하여 대장균에 감염시킨 뒤 원심분리 하였더니 대장균에서 발견된 것은 DNA 였다. 즉, 유전적인 정보는 DNA 가 전달한다는 것을 밝혀내었다.

The Nature of The Gene

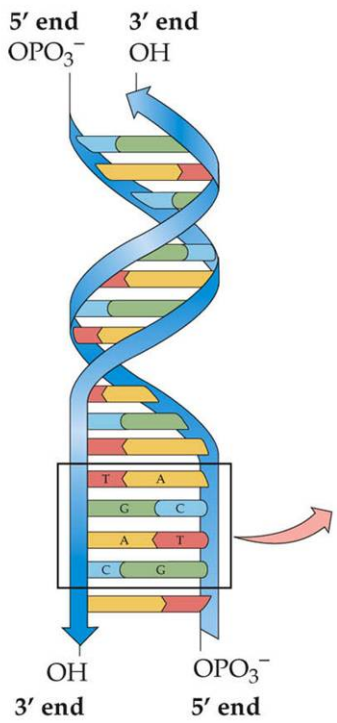
Watson and Crick (1953)

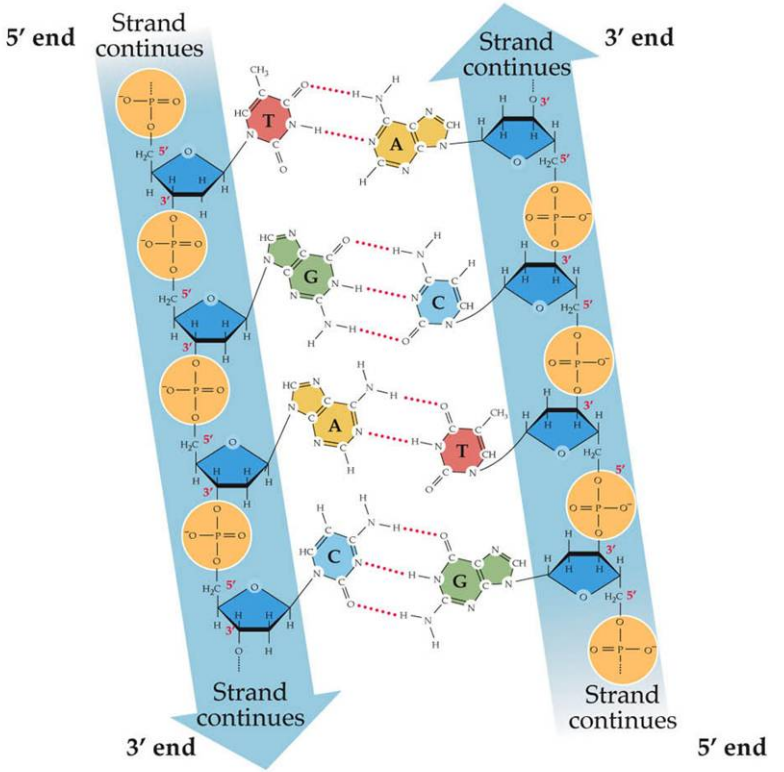


- 1953년 Crick 과 Watson 은 DNA 의 구조적 모델을 제안하였다.
- 이 제안은 화학자 Chargaff가 purine (Adenine, Guanine) 과 pyrimidine (Thymine, Guanine)의 비율이 1:1임을 밝힘으로써 가능했다
- 또한 X-ray 촬영기사인 Franklin 과 Wilkins 의 자료 제공으로 이 연구가 행해질 수 있었다.
- 이 둘은 1962년 노벨상을 수상하였다.

The Nature of The Gene

DNA Structure





LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 11.7 Base Pairing in DNA Is Complementary
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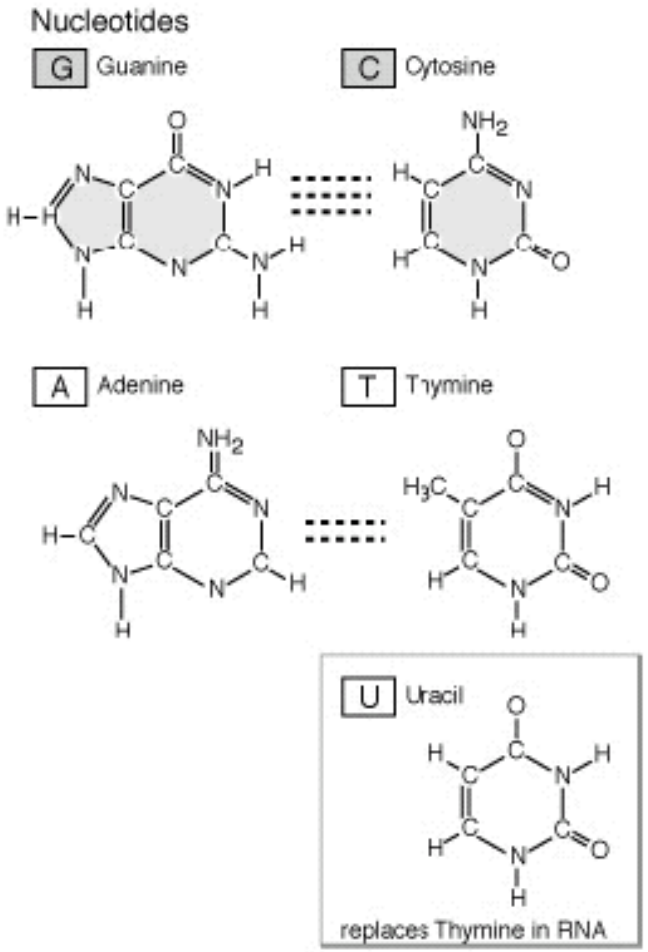


DNA 의 이중나선 구조
Adenine 은 Thymine 과 결합하게 되고 Guanine 은 Cytosine 과 결합하게 된다.
오른쪽 그림에서 DNA에서 RNA 로 transcription이 일어날 때에 Thymine 은 RNA 에서 Uracil 의 형태로 존재하게 된다.

The Nature of The Gene

DNA Structure

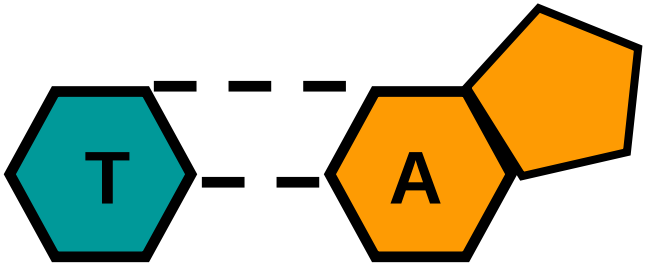
Purines	Pyrimidines	Base Pairs	#of H-Bonds
Adenine (A)	Thymine (T)	A = T	2
Guanine (G)	Cytosine (C)	C ≡ G	3

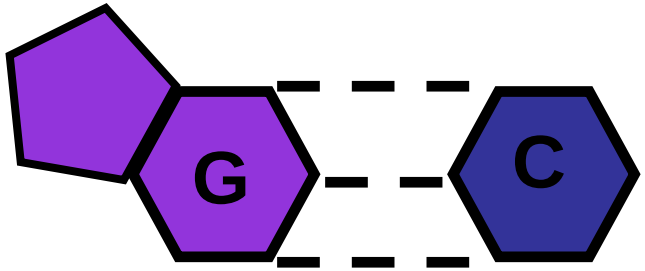
Adenine must pair with **Thymine**

- **Guanine** must pair with **Cytosine**

– Adenine 은 Thymine 과만 이중결합을 하며

– Guanine 은 cytosine 과 삼중결합을 하게 된다.





The Nature of The Gene

DNA Structure

- **Question**

  If there is 30% Adenine, how much Cytosine is present?

The Nature of The Gene

DNA Structure

- There would be 20% Cytosine.

Adenine (30%) = Thymine (30%)

Guanine (20%) = Cytosine (20%)

(50%) = (50%)

The Nature of The Gene

- **Scientific foundation of modern biotechnology**
  - Discovery and understanding of nucleic acids
  - Enzymatic tools required for DNA manipulation
    - Restriction endonucleases from bacteria in 1970

    (DNA 사슬을 원하는 부위에서 끊을 수 있는 효소가 발견되었고)
    - Development of plasmid technology in 1973

    (Plasmid technology 가 개발되어 원하는 유전적인 정보를 다른 개체에서 발현할 수 있게 되었다)
    - Ligases, enzymes that join the ends of two DNA molecules together

    (DNA 를 서로 붙일 수 있는 효소를 발견하였다)
  - Accumulated knowledge of cell structure, biochemistry, and heredity

Proteins & Genes

Amino Acid, Peptide & Protein

- **Amino acid**: a compound that contains both an amino group(- NH_2) and a carboxyl group(- COOH)

- **Peptide**: the name given to a short polymer of amino acids; they are classified by the number of amino acids in the chain
  - dipeptide: a molecule containing two amino acids joined by a peptide bond
  - tripeptide: a molecule containing three amino acids joined by peptide bonds
  - polypeptide: a macromolecule containing many amino acids joined by peptide bonds

- **Protein**: a biological macromolecule of molecular weight 5000 g/mol of greater, consisting of one or more polypeptide chains

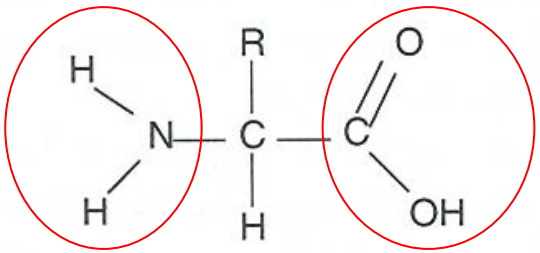


Fig. 13.3 The basic structure of an amino acid consists of an amino group ($-NH_2$), a carboxyl group ($-COOH$), and a side chain (R). The R group gives each amino acid its distinguishing physico-chemical properties, e.g., charged, uncharged, acidic, and basic.

Proteins & Genes

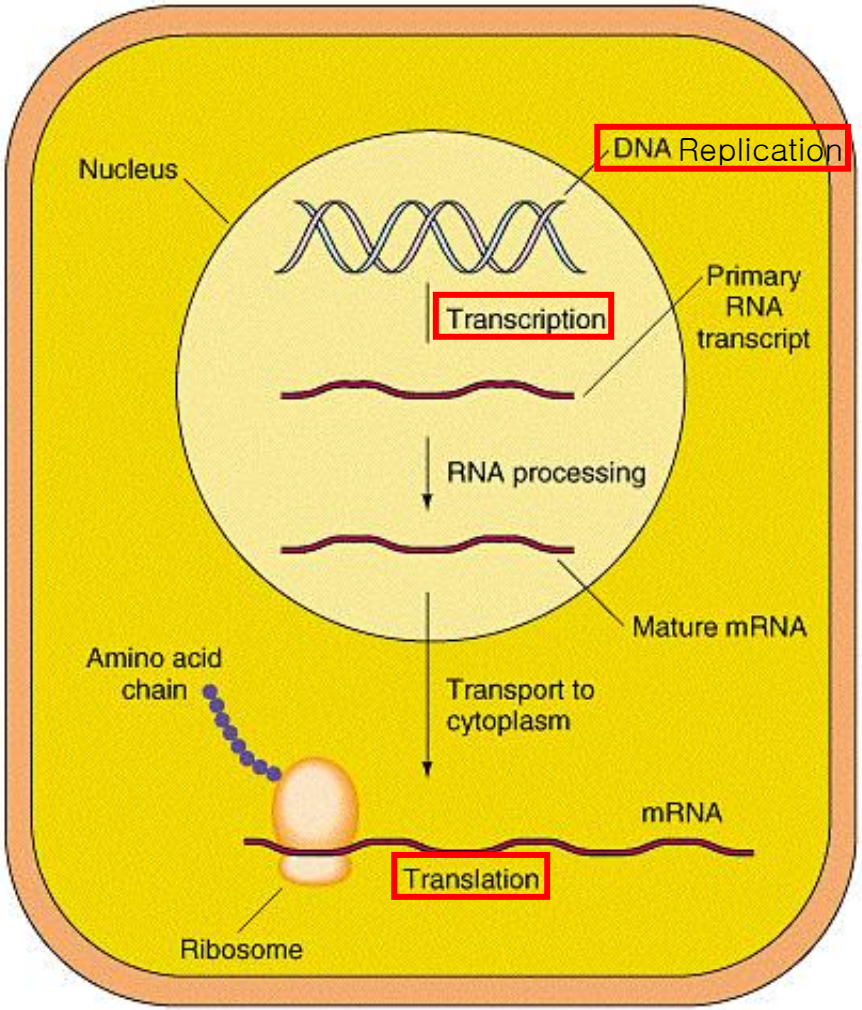
Amino Acids

TABLE 13.2 Amino Acids in Proteins

Amino acid	Three-letter code	One-letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Proteins & Genes

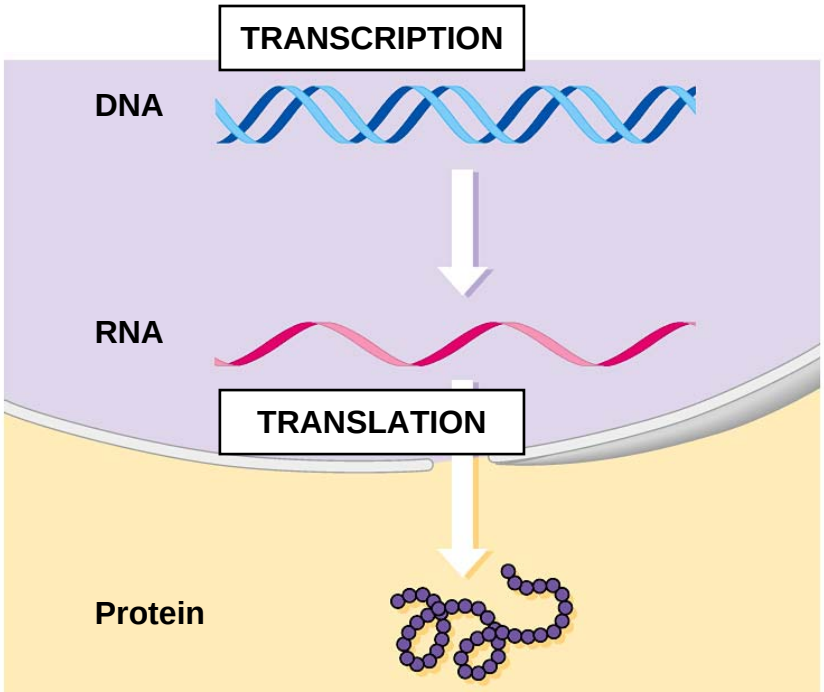
Central Dogma: from gene to protein



1. Replication
   - Duplication of DNA using DNA as the template
2. Transcription
   - Synthesis of RNA using DNA as the template
3. Translation
   - Synthesis of proteins using RNA as the template

Proteins & Genes

Ribonucleic Acid (RNA)



- **Compared to DNA**
  - The base Uracil(U) substitutes for Thymine(T) and pairs with Adenine(A)
  - Single stranded except short double-stranded regions
  - Much shorter than DNA
  - Present only transiently, and degraded
  - mRNA (encode amino acid sequence), tRNA (adapter carrying amino acid to the site of protein synthesis), rRNA (part of ribosome, work bench of protein synthesis)

Biotechnologies

- Recombinant DNA technologies
  DNA 합성기술
- Electrophoresis 전기영동
- PCR
- Others (Blottings etc.)

Recombinant DNA Technologies

What is Recombinant DNA?

- **Recombinant DNA**: A DNA molecule produced *in vitro* by genetic recombination; the exchange of genes between two DNA molecules to form new combinations of genes on one molecule of DNA.

- **Vector**: A self replicating DNA molecule, e.g., a plasmid, used to carry a gene from one organism to another.
  - **Plasmid**: Small, mobile piece of DNA found in bacteria that, for example, confers antibiotic resistance, used in genetic engineering. Plasmids are separate from the bacterial chromosome but still multiply during cell growth.

Recombinant DNA Technologies

- Why transfer a gene from one organism to another?
  - To get a gene product, e.g., insulin

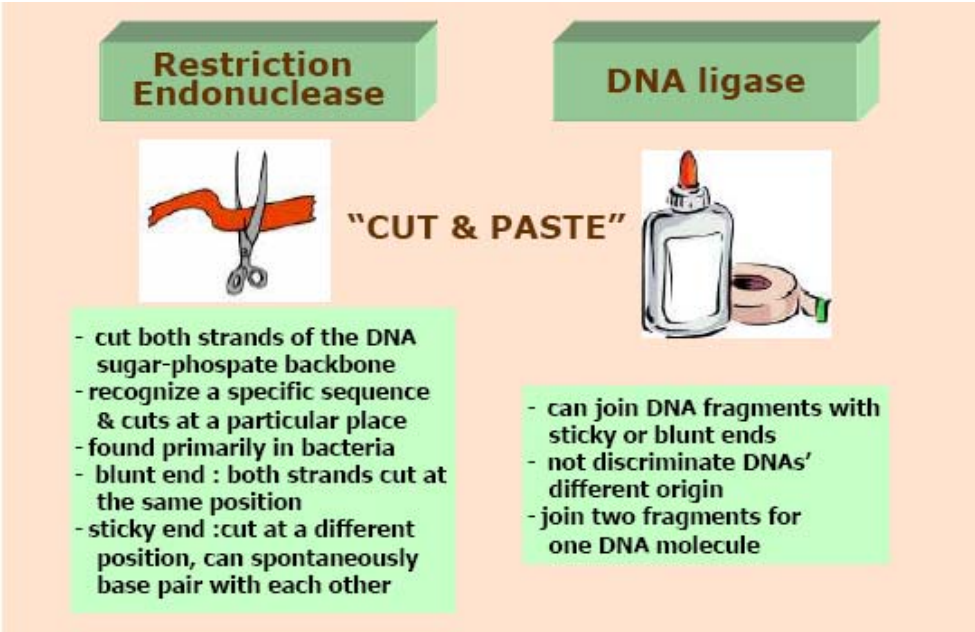
    인슐린에 관한 유전정보를 가진 vector 를 다른 생물체에 주입하게 되면 vector가 생존하고 자기 복제하면서 insulin 을 생산하게 된다

  - To get a genetically modified organism, e.g., a genetically engineered *Rhizobium* has enhanced nitrogen fixation 뿌리혹 박테리아

  - To isolate a gene and obtain large quantities of it for nucleotide sequence analysis

Recombinant DNA Technologies

Restriction Endonuclease & DNA ligase



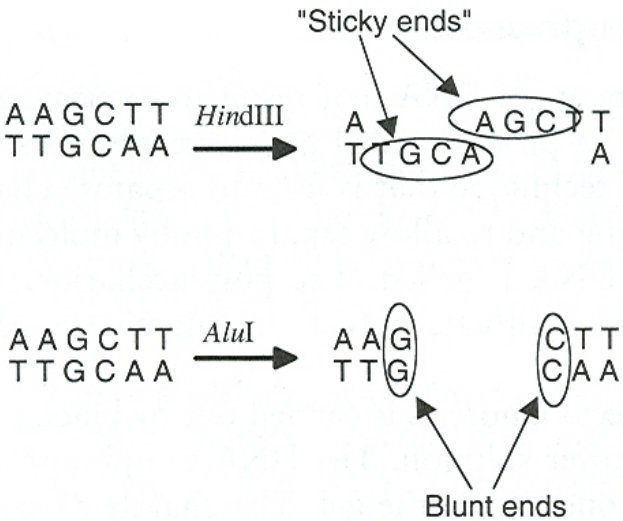
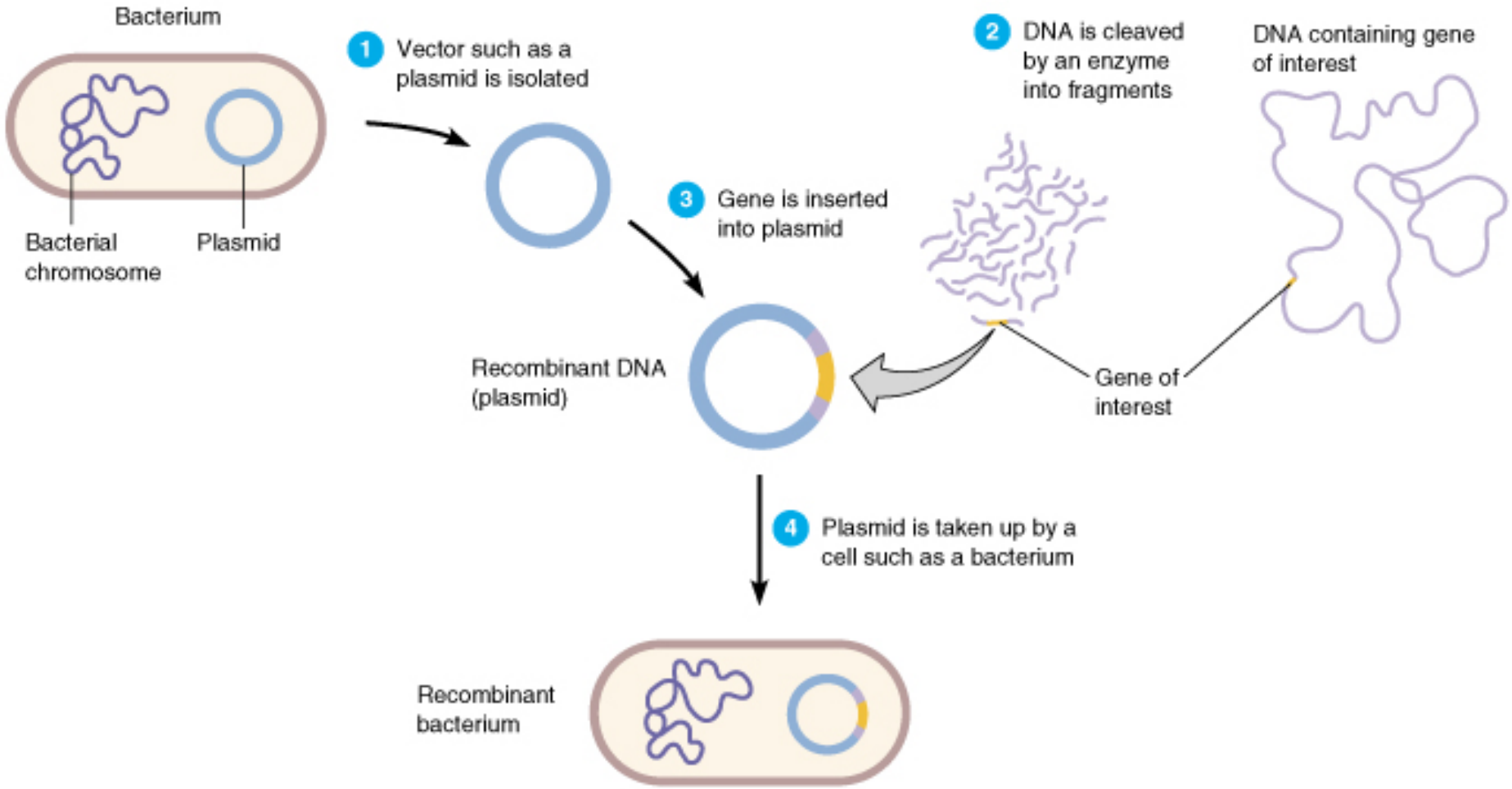


Fig. 13.7 Restriction enzymes are used to cut specific sequences of DNA. In this example, *Hin*dIII cuts the following sequence between the two As and leaves "sticky ends" that consist of single strands of DNA. *Alu*I leaves blunt ends because no lengths of single strands are formed by the cut through the DNA.



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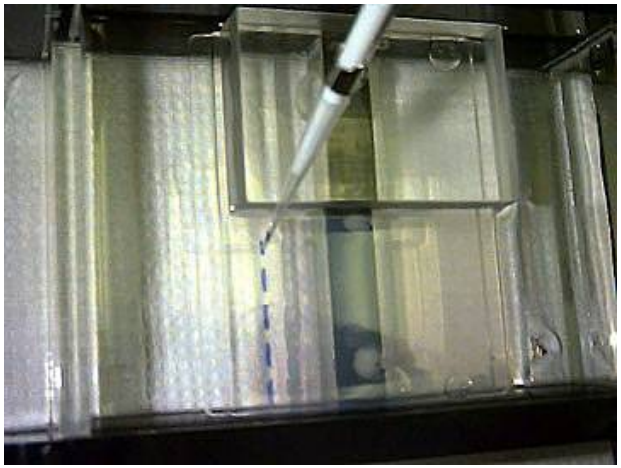
Electrophoresis

- **Electrophoresis**: A biochemical technique that is used to separate charged molecules in solution.
  - For ease of handling and to allow separation by molecule size, the aqueous solution used to separate DNA is gelled
  - A current is applied so that the negative charged DNA molecules migrate towards the positive electrode and is separated by fragment size

Electrophoresis

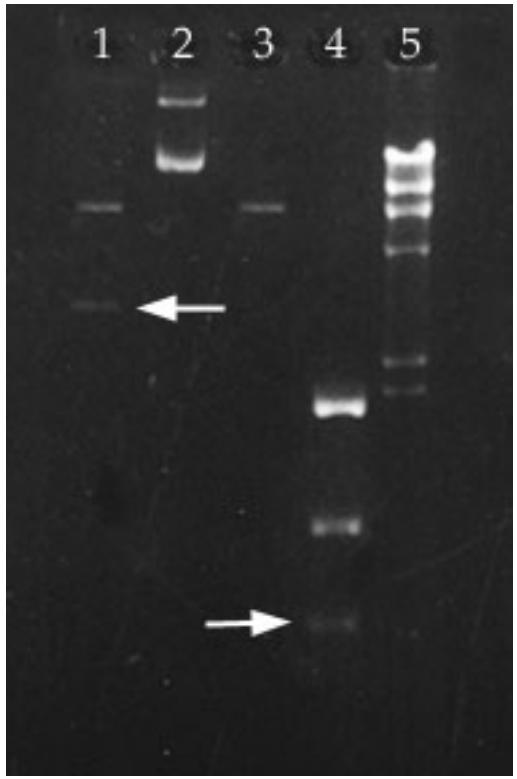
① Preparing DNA sample



② Loading sample

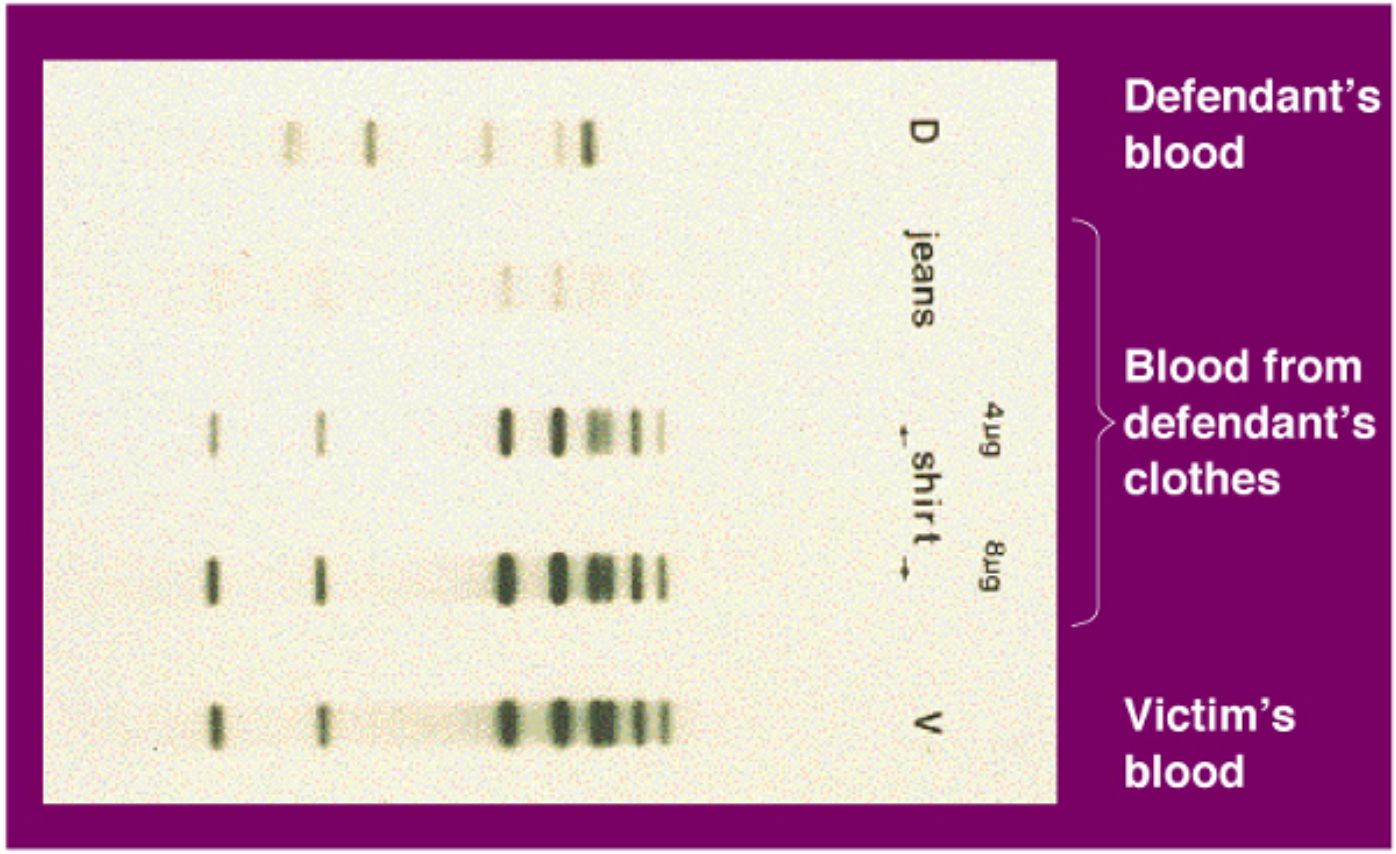
③ Connect to a power supply and running

④ Use UV to visualize DNA (UV florescence dye was added before loading)



⑤ Result

Electrophoresis



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Forensics applications:

examining DNA markers to identify criminals

PCR(Polymerase Chain Reaction)

- It is hard to exaggerate the impact of the polymerase chain reaction. PCR, the quick, easy method for generating unlimited copies of any fragment of DNA, is one of those scientific developments that actually deserves timeworn superlatives like "revolutionary" and "breakthrough."

- Tabitha M. Powledge

Polymerase Chain Reaction

Purpose of PCR

- Amplify specific nucleic acids in vitro ("Xeroxing" DNA)
- PCR will allow a short stretch of DNA (usually fewer than 3000 base pairs) to be amplified to about a million fold
- This amplified sample then allows for size determination and nucleotide sequencing
- Introduced in 1985 by Kary Mullis
- Millions of copies of a segment of DNA can be made within a few hours.

Polymerase Chain Reaction

Three Steps of PCR

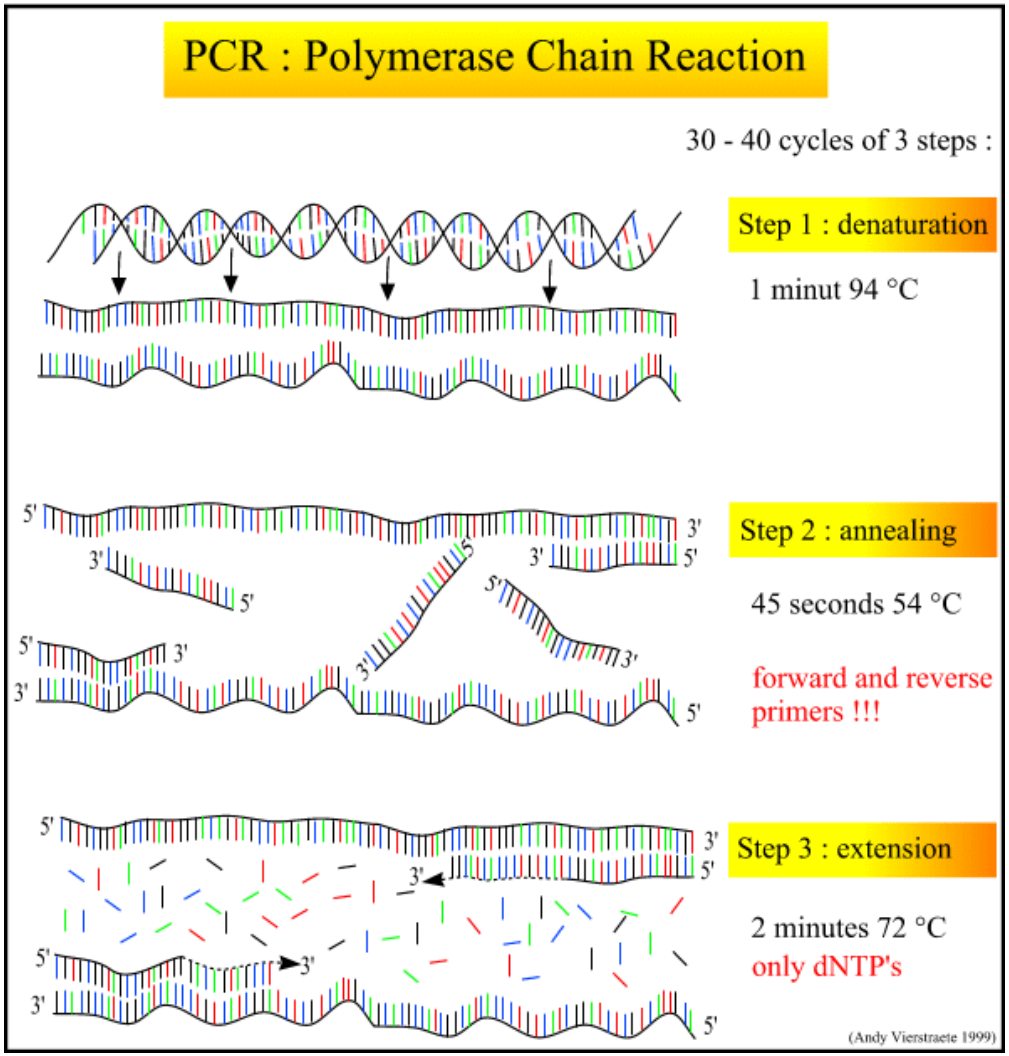
Three steps of PCR

1. Denaturation: Double Stranded DNA is denatured by heat into single strands.
2. Annealing: Short Primers for DNA replication are added to the mixture.
3. Extension: DNA polymerase catalyzes the production of complementary new strands.

- Copying The process is repeated for each new strand created
- All three steps are carried out in the same vial but at different temperatures

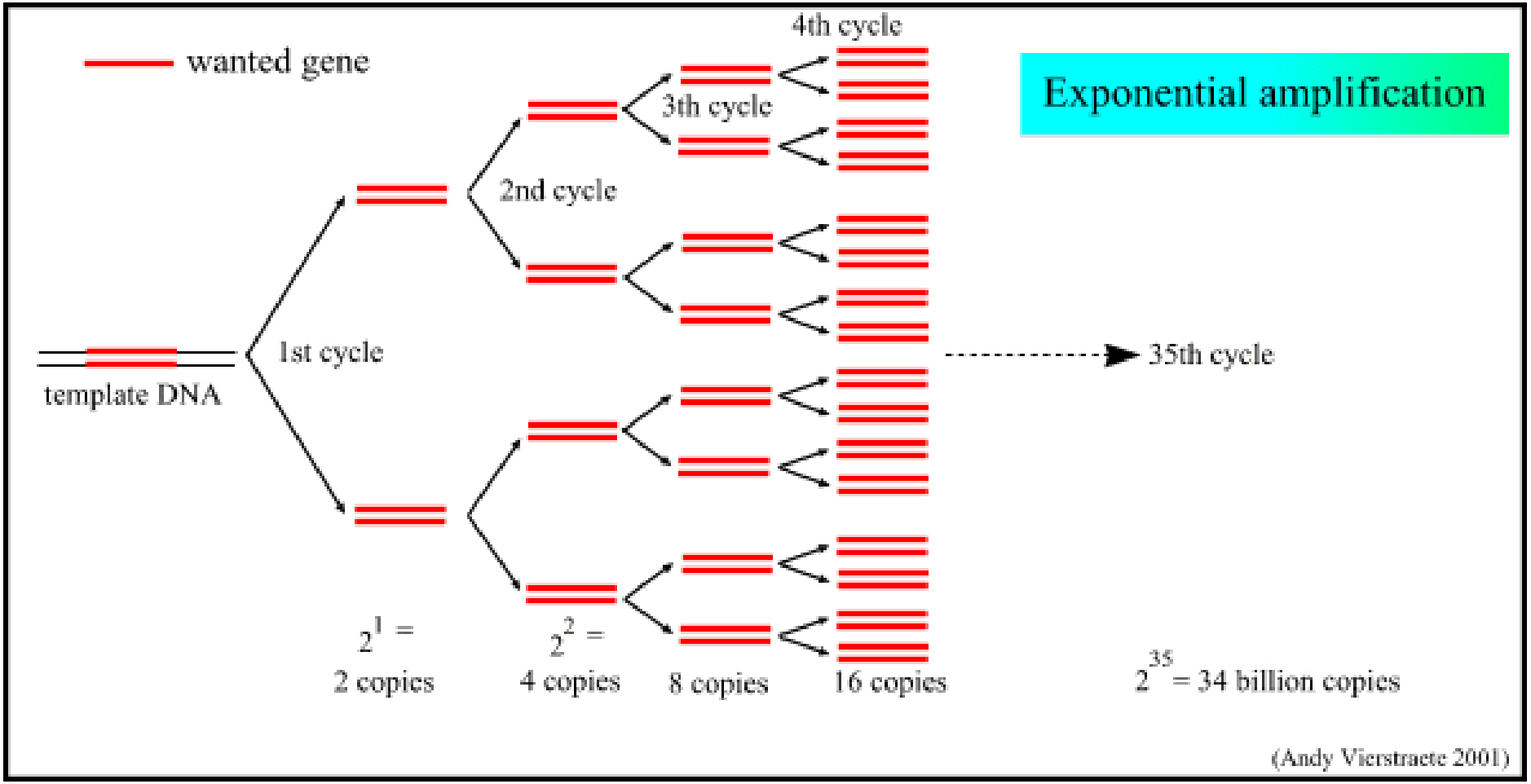
Polymerase Chain Reaction

Three Steps of PCR

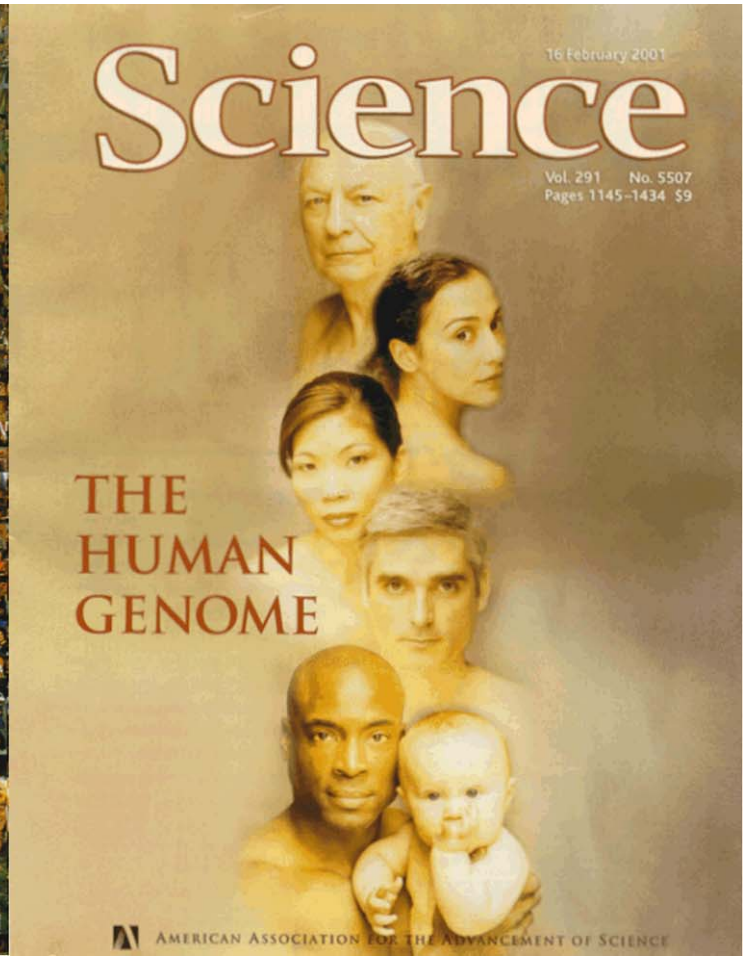


Polymerase Chain Reaction

PCR amplification



Human Genome Project



Human Genome Project

June 2000

Craig Venter

CELERA

Francis Collins

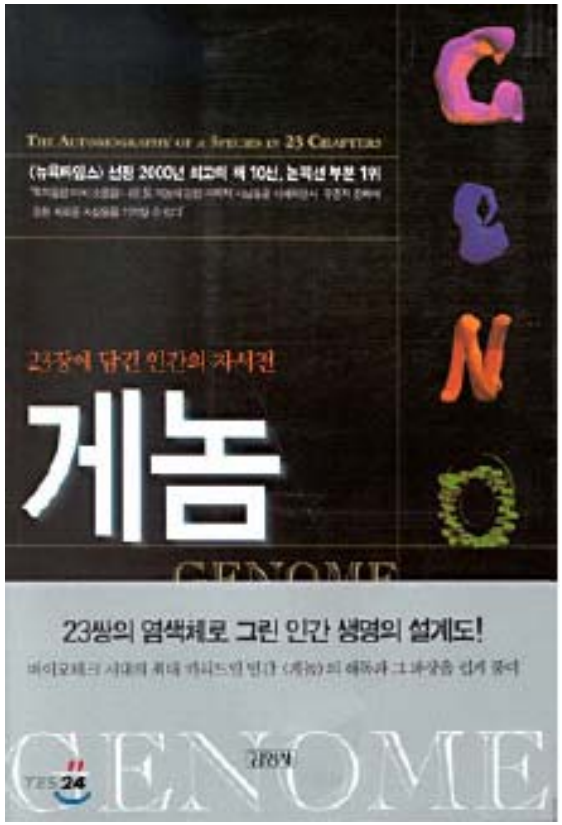
http://www.nhgri.nih.gov/

Stock value:

June 1st 2000:	63.50
June 30th 2000:	92.00
February 19th 2004:	14.94

Human Genome Project

- Book consists of:
  - 3 billion base pairs=letters
  - 1 billion triplet codes(codon)=words
  - 24 chromosomes = chapters
  - Exons = paragraphs
  - Introns= adverts



GENOME

By Matt Ridley

Human Genome Project

Brief History

- Proposed in 1985
- 1988. Initiated and funded by NIH and US Dept. of Energy ($3 billion set aside)
- 1990. Work begins.
- 1998. Celera announces a 3-year plan to complete the project years early
- Published in Science and Nature in February, 2001
- Completed 2003

Human Genome Project

Goals of HGP

Goals of HGP

- 인간 유전자의 지도를 파악하는 것과
- 30억개에 이르는 base pair 의 순서를 결정하는 것,
- 인간의 인종별로, 개인별로 다른 유전자를 찾아 내는 것이고
- 인간 이외의 몇몇 생물체의 유전자 지도를 작성 하는 것이었다.

Human Genome Project

Goals of HGP (cont'd)

Other Goals of HGP

- 그리고 더불어 이러한 분석을 가능하게 하는 기반 기술들의 개발도 목적이 있었고
- 인간 유전자의 정보를 공개하는 것과
- 이 연구의 윤리적, 법적, 사회적 논의 들을 연계시키기 위한 것이었다.

Human Genome Project

Public Project

- International Human Genome Mapping Consortium (HGP)
- 고전적인 방법으로
- Vector 에 인간의 유전자를 쪼개어 넣고 vector 를 증식시켜 인간의 유전자를 복제한다
- 그리고 복제된 유전자를 분석하는 작업을 차근차근 DNA 의 순서대로 진행한다

Human Genome Project

Private project

- Celera Genomics
- Used Shotgun Technology
- 이 방법은 유전자를 일단 잘게 조각 낸 뒤 이들을 각각 해석하고 이 해석된 data를 슈퍼컴퓨터에서 퍼즐을 맞추듯이 재구성 하는 방식이다
- 이 방법으로 획기적인 시간의 단축을 가져와 HGP를 압박하기에 이르렀고 당시 대통령인 클린턴의 중재로 2001년 같은 날에 발표를 하게 된다.

Human Genome Project

Scientific vs Commercial Goals

- “The HGP's commitment from the outset was to create a scientific standard (an entire reference genome). Most private-sector human genome sequencing projects, however, focused on gathering just enough DNA to meet their customers' needs—probably in the 95% to 99% range for gene-rich, potentially lucrative regions. Such private data continue to be enriched greatly by accurate free public mapping (location) and sequence information. Celera's shotgun sequencing strategy, for example, created millions of tiny fragments that had to be ordered and oriented computationally using HGP research results. Most data at Celera, Incyte, and other genomics information-based companies are proprietary or available only for a fee.”