Biotechnology

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

    If one inject vector that has genetic information about insulin to the other living organisms, insulin was produced by replication from survival vector.

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

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

Recombinant DNA Technologies

Restriction Endonuclease & DNA ligase

Restriction Endonuclease

- cut both strands of the DNA sugar-phospate backbone
- recognize a specific sequence & cuts at a particular place
- found primarily in bacteria
- blunt end : both strands cut at the same position
- sticky end :cut at a different position, can spontaneously base pair with each other

"CUT & PASTE"

DNA ligase

- can join DNA fragments with sticky or blunt ends
- not discriminate DNAs' different origin
- join two fragments for one DNA molecule

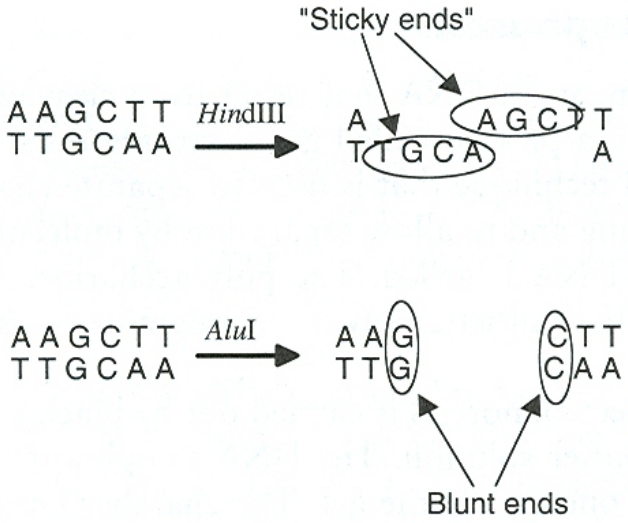
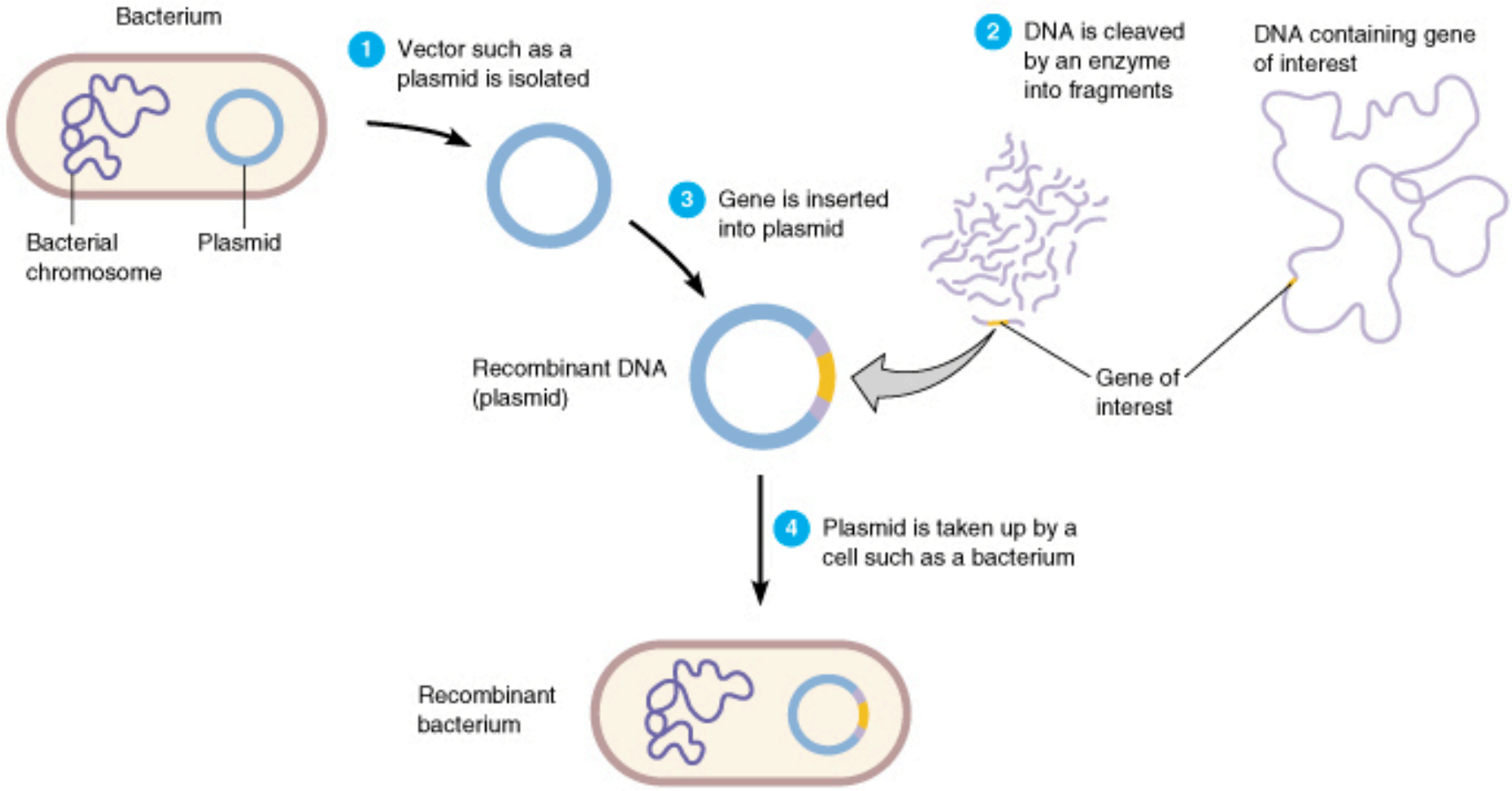


Fig. 13.7 Restriction enzymes are used to cut specific sequences of DNA. In this example, *Hind*III cuts the following sequence between the two As and leaves "sticky ends" that consist of single strands of DNA. *Alu*I leaves blunt ends because no lengths of single strands are formed by the cut through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

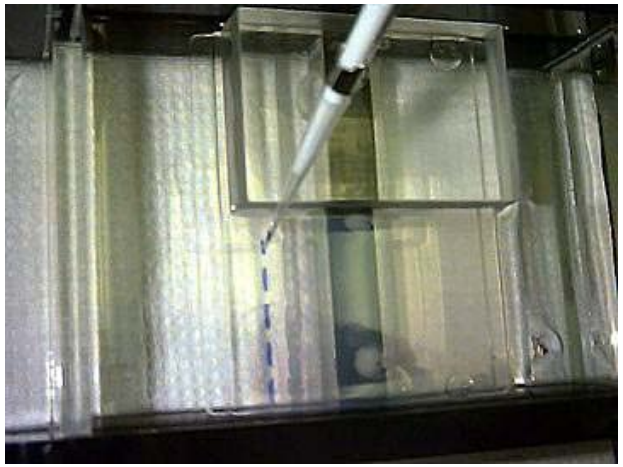


Electrophoresis

- **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

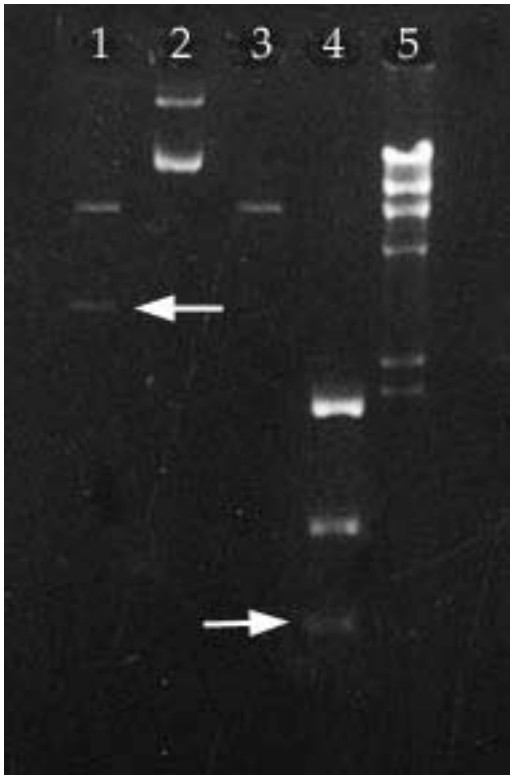
① Preparing DNA sample



② Loading sample

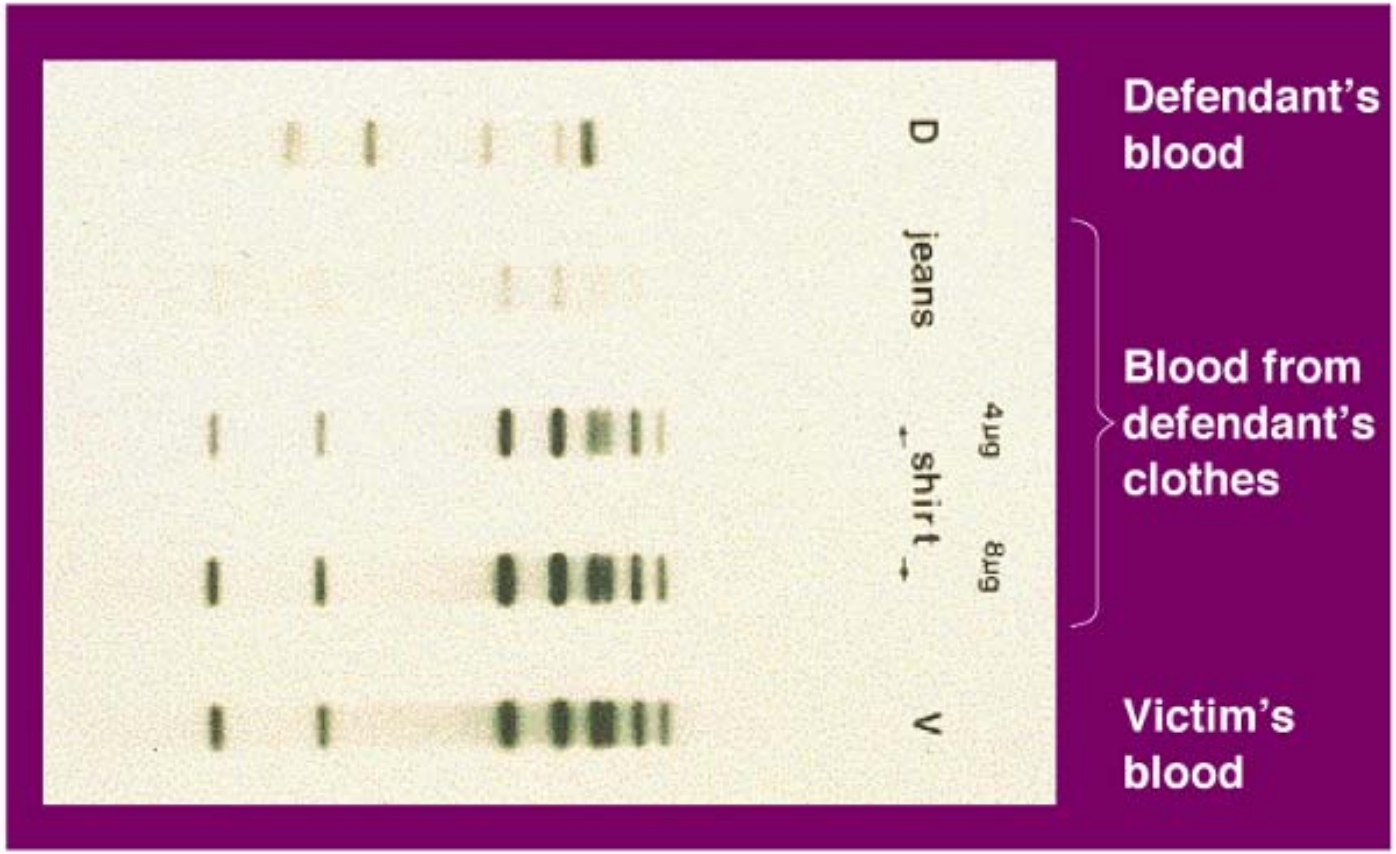
③ Connect to a power supply and running

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

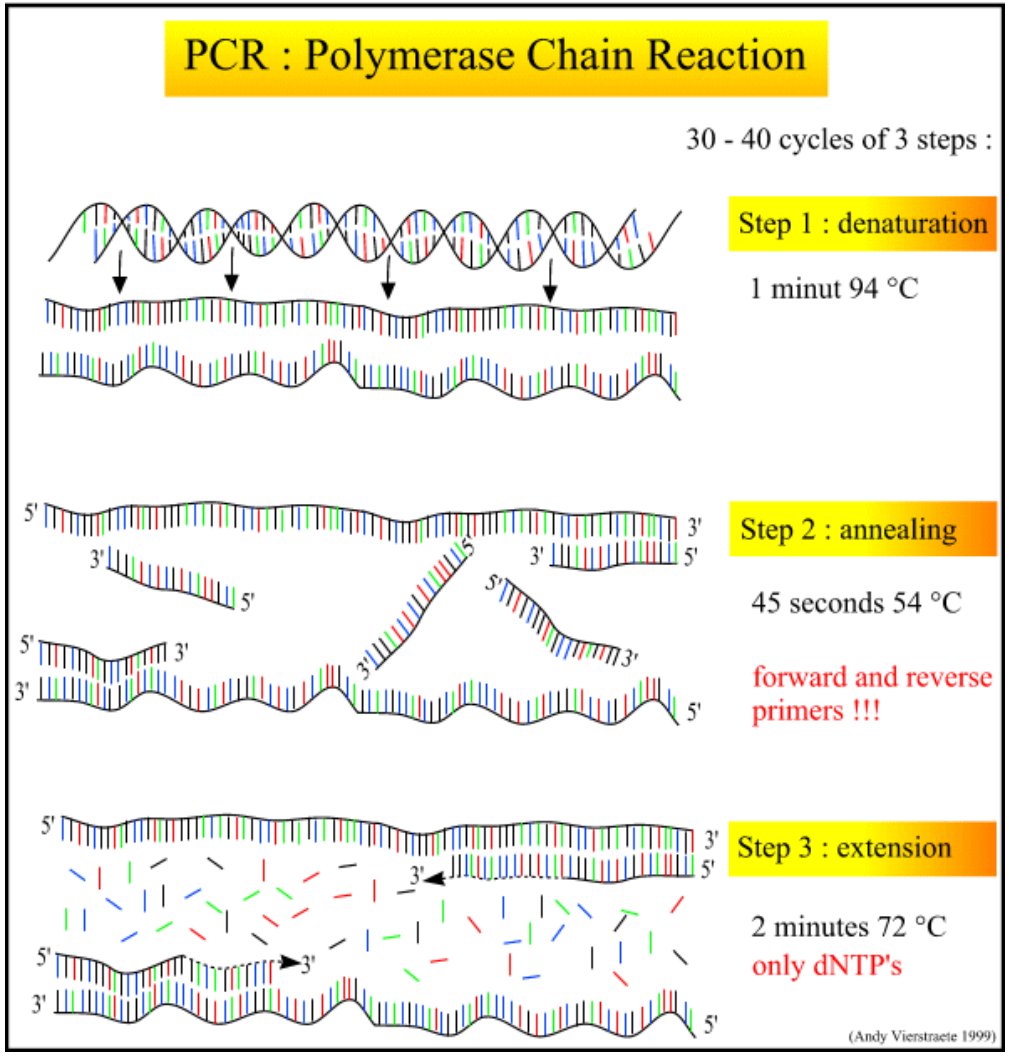
Three steps of PCR

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

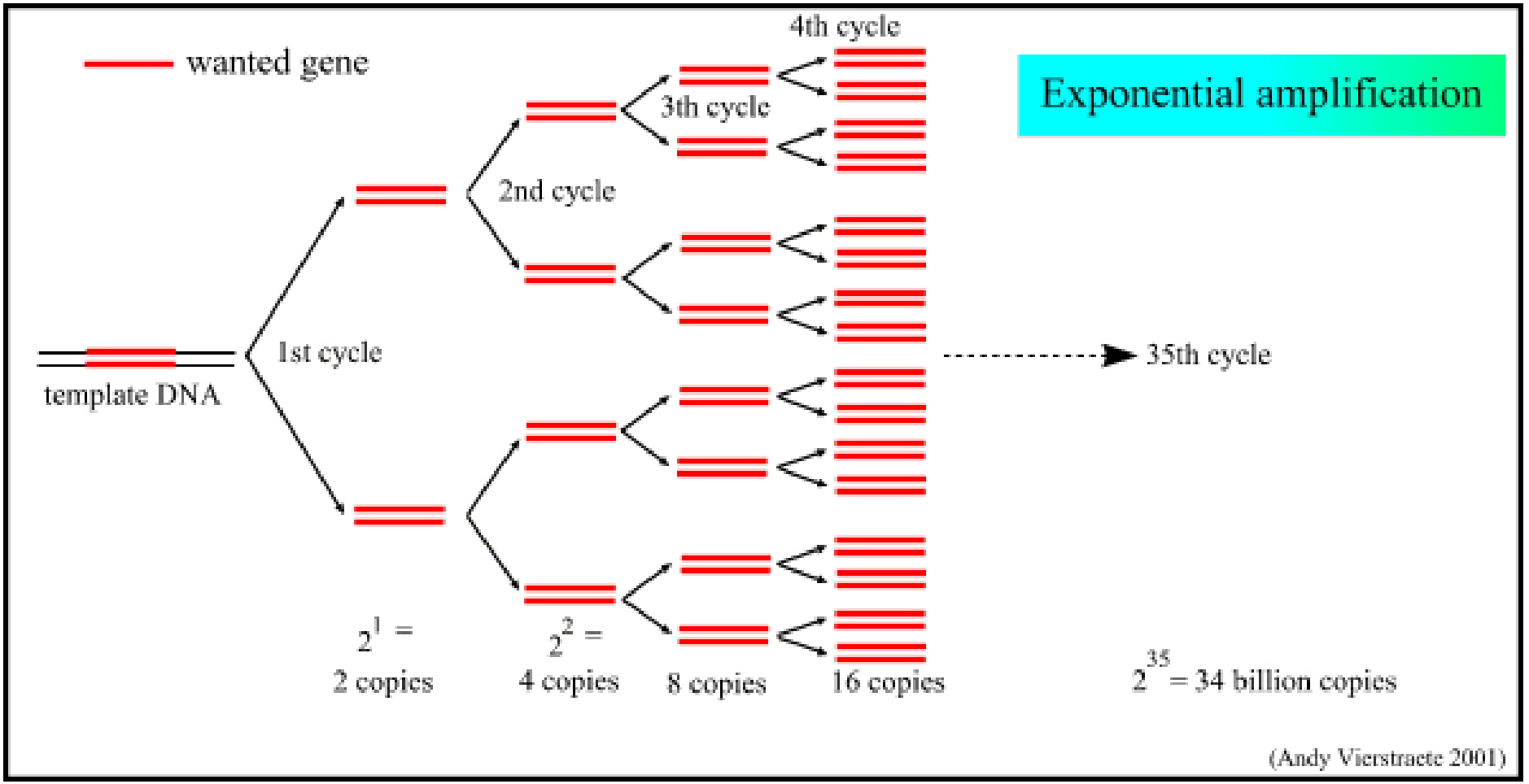
Polymerase Chain Reaction

Three Steps of PCR



Polymerase Chain Reaction

PCR amplification



Human Genome Project

Human Genome Project

June 2000

Craig Venter

Francis Collins

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

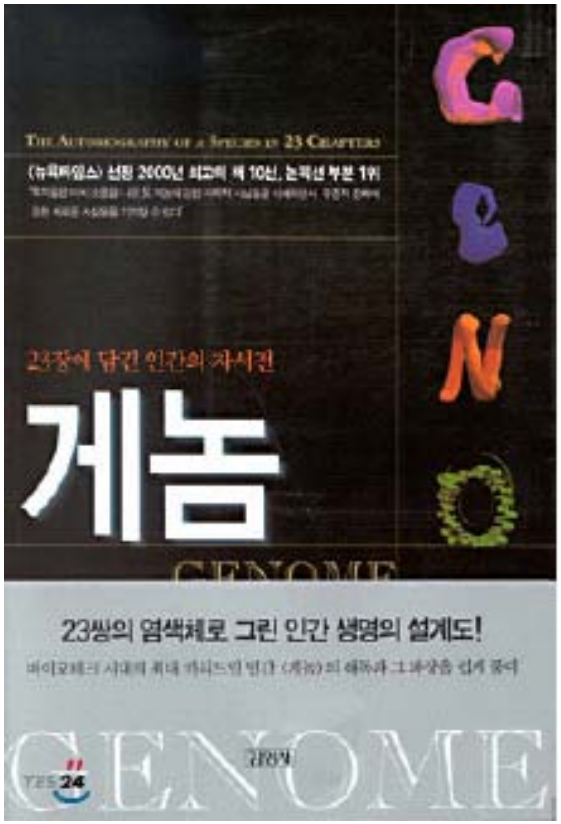
genome.gov
National Human Genome Research Institute
Advancing human health through genetic research

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

- Find the genetic map of the human
- Determine the sequence of 3 billion chemical ba se pairs
- Find the difference genes between the races and individuals
- Find the genetic map of some kind of other living organisms

Human Genome Project

Goals of HGP (cont'd)

Other Goals of HGP

- Develop the basic techniques that enable gene analysis
- Open the human genetic information to the public
- Linked with not only information on genetic conditions, but ethical, legal, and social issues.

Human Genome Project

Public Project

- International Human Genome Mapping Consortium (HGP)
- In classical method, vectors were used to copy genes and other pieces of chromosomes to generate enough identical genes.
- Then analyze the cloned gene one by one in regular sequence of DNA.

Human Genome Project

Private project

- Celera Genomics
- Used Shotgun Technology, to produce DNA fragments.
- Using several complex alignment algorithims and a supercomputer, the pieces were combined and the genome was reassembled.
- In 2001, at the time of the joint publications, press releases announced that the project had been completed by both groups.

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.”