Teknik Analisis Biologi Molekuler dan Aplikasinya

Kuliah Pengantar TABM

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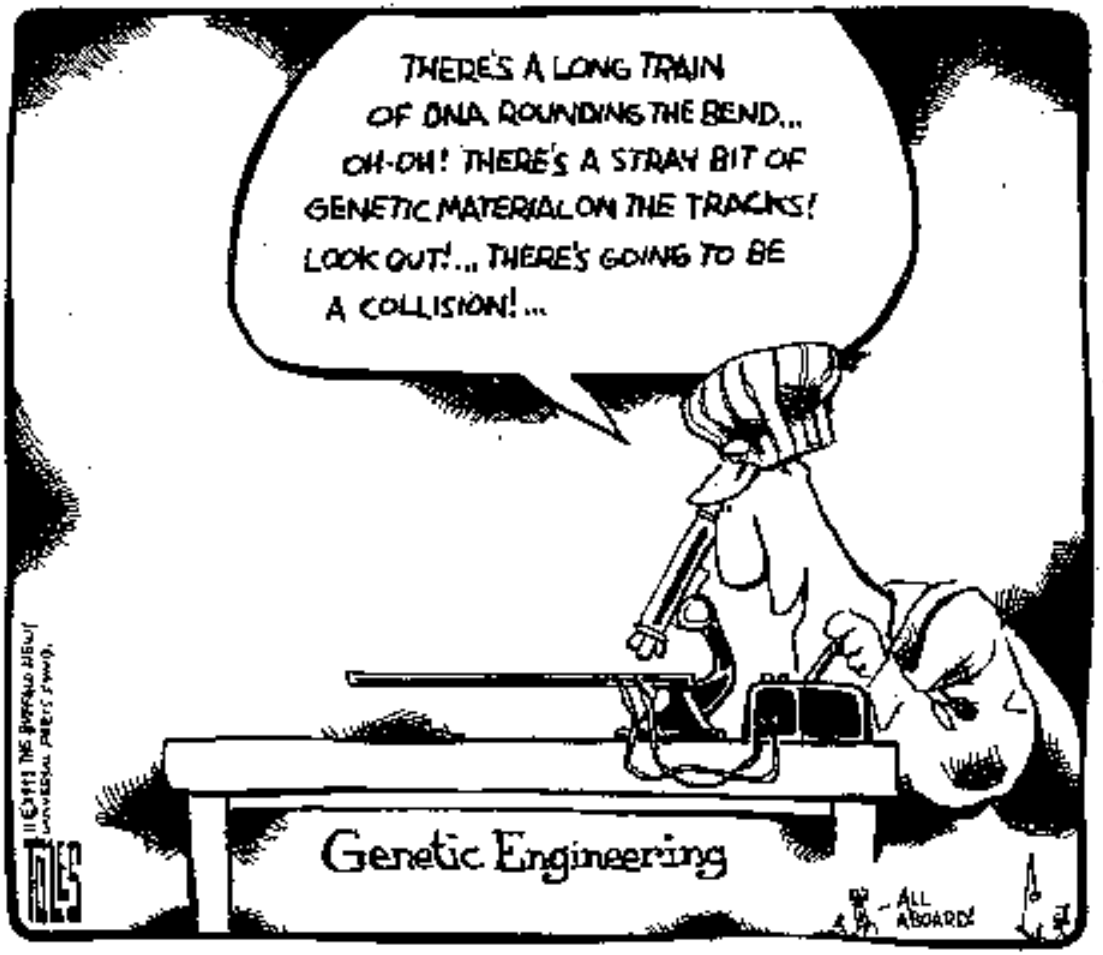
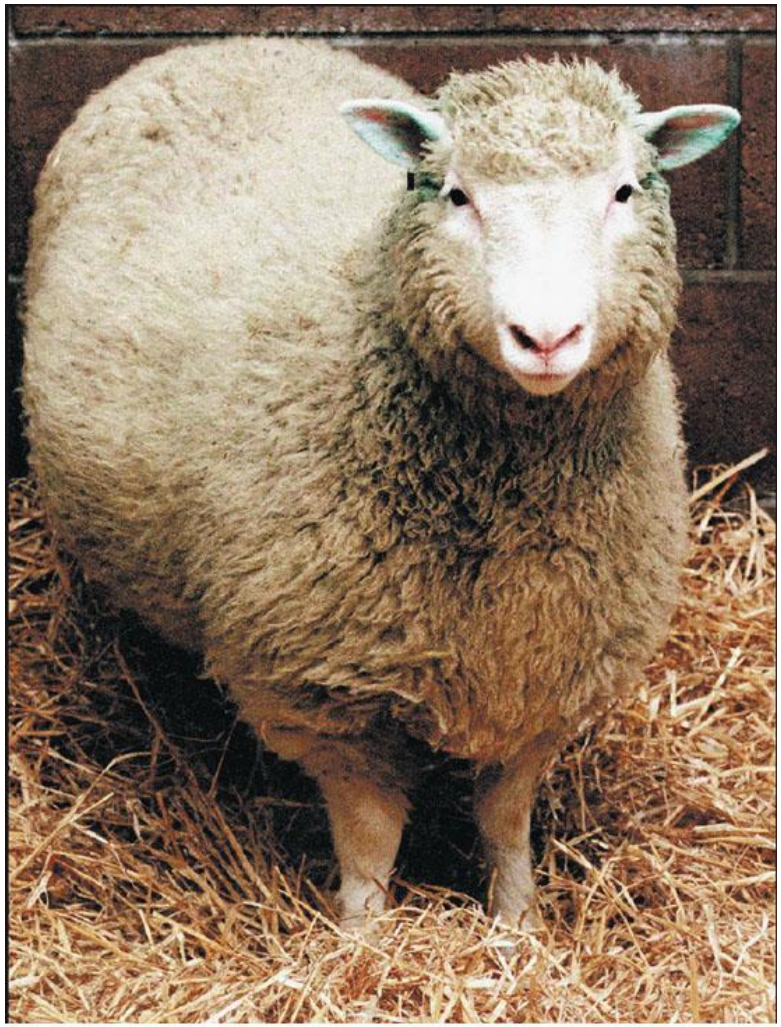
ISO /SNI 17025:2005 and ISO 9001/2008

Biotechnology and Recombinant DNA



The late Dolly, the most famous sheep in the world, produced by cloning techniques.



p.331

Biotechnology

- The use of microorganisms, cells, or cell components to make a product
  - Foods
  - Vaccines
  - Antibiotics
  - Vitamins
  - Biodegradation
- Selective breeding

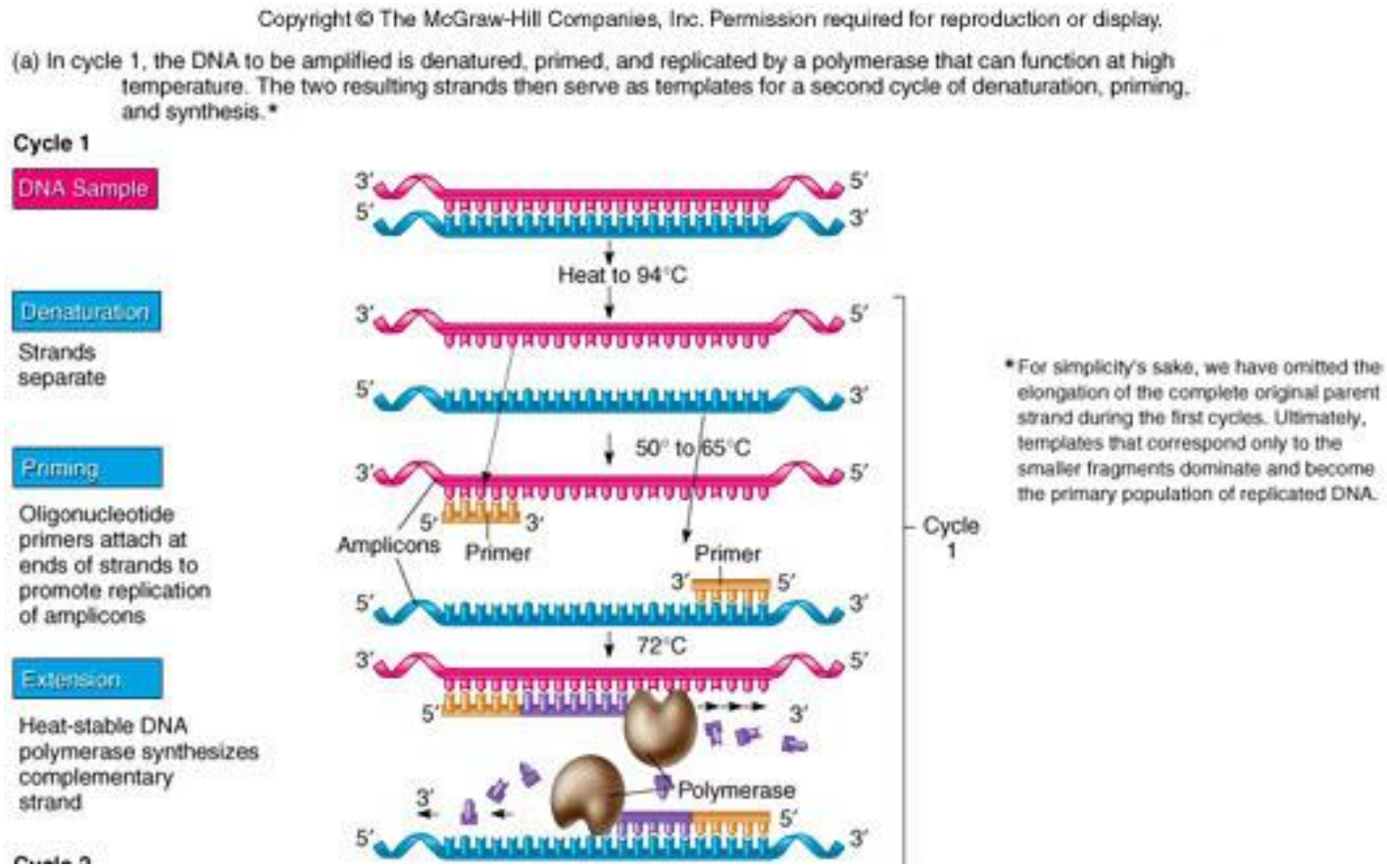
Useful Properties of DNA

- DNA sequences specify gene locations and protein amino acid sequence
- Restriction endonucleases cut at specific nucleotides; size of pieces gives us information about DNA sequence
- Nucleotides hydrogen bond with complementary nucleotides
- DNA hybridization allows recognition of specific genes

In-Situ Hybridization

- Target nucleic acid found in intact cells.
- Provides information about presence of specific DNA targets and distribution in tissues.
- Probes must be small enough to reach nucleic acid.
- Radioactive or fluorescent tags used.
- Requires experience.

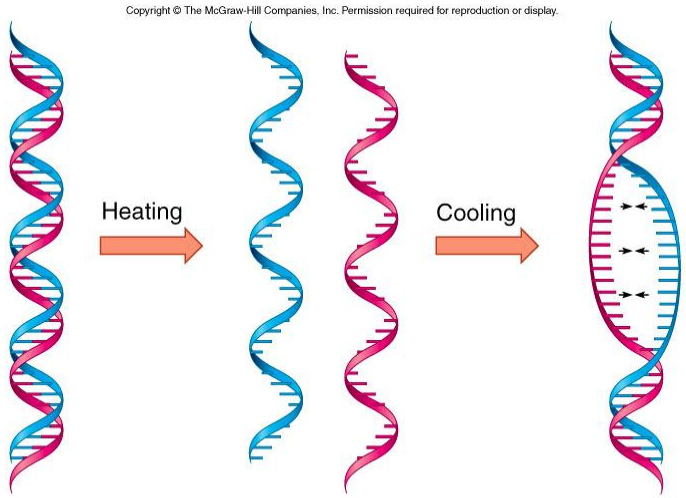
Polymerase Chain Reaction (PCR) Amplifies DNA



- Primers specify what DNA is copied

Useful Properties of DNA

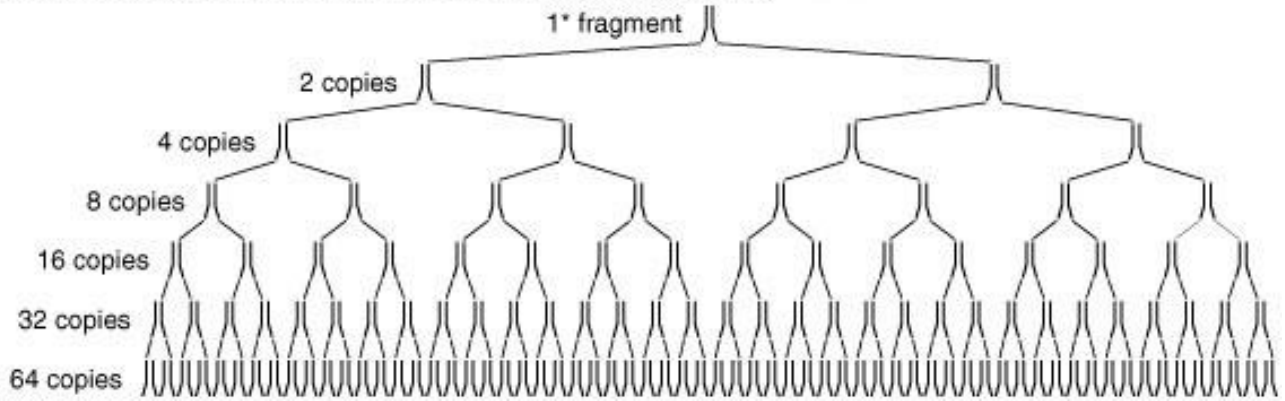
- The complementary strands of DNA can be separated and re-associated by heating and cooling
- One strand of DNA specifies the sequence of the other strand
  - mRNA specifies the sequence of the gene (DNA)



(a) DNA heating and cooling. DNA responds to heat by denaturing—losing its hydrogen bonding, and thereby separating into its two strands. When cooled, the two strands rejoin at complementary sites. The two strands need not be from the same organisms as long as they have matching sites.

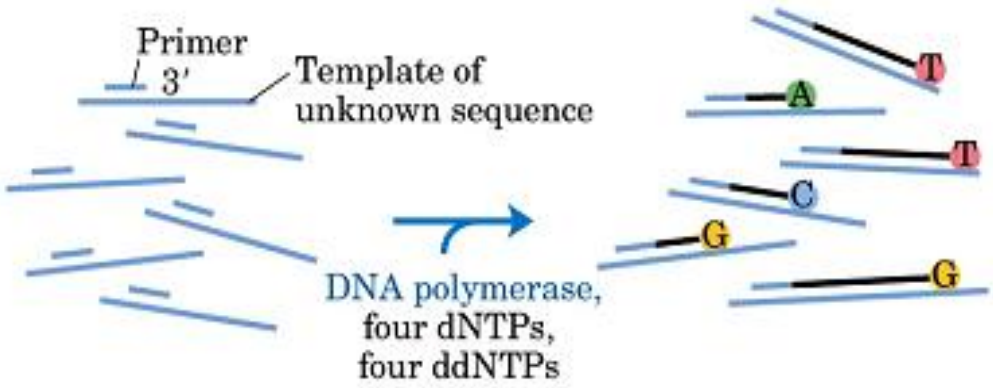
PCR Amplifies DNA

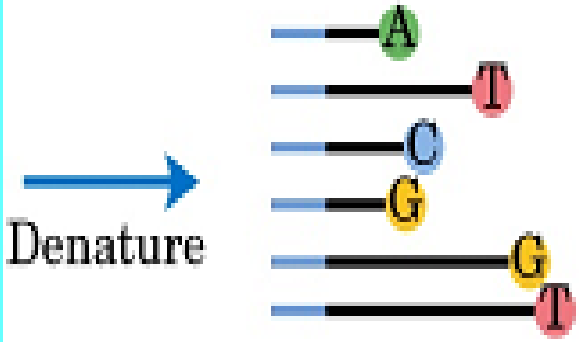
(b) A view of the process after 6 cycles, with 64 copies of amplified DNA. Continuing this process for 20 to 40 cycles can produce millions of identical DNA molecules.



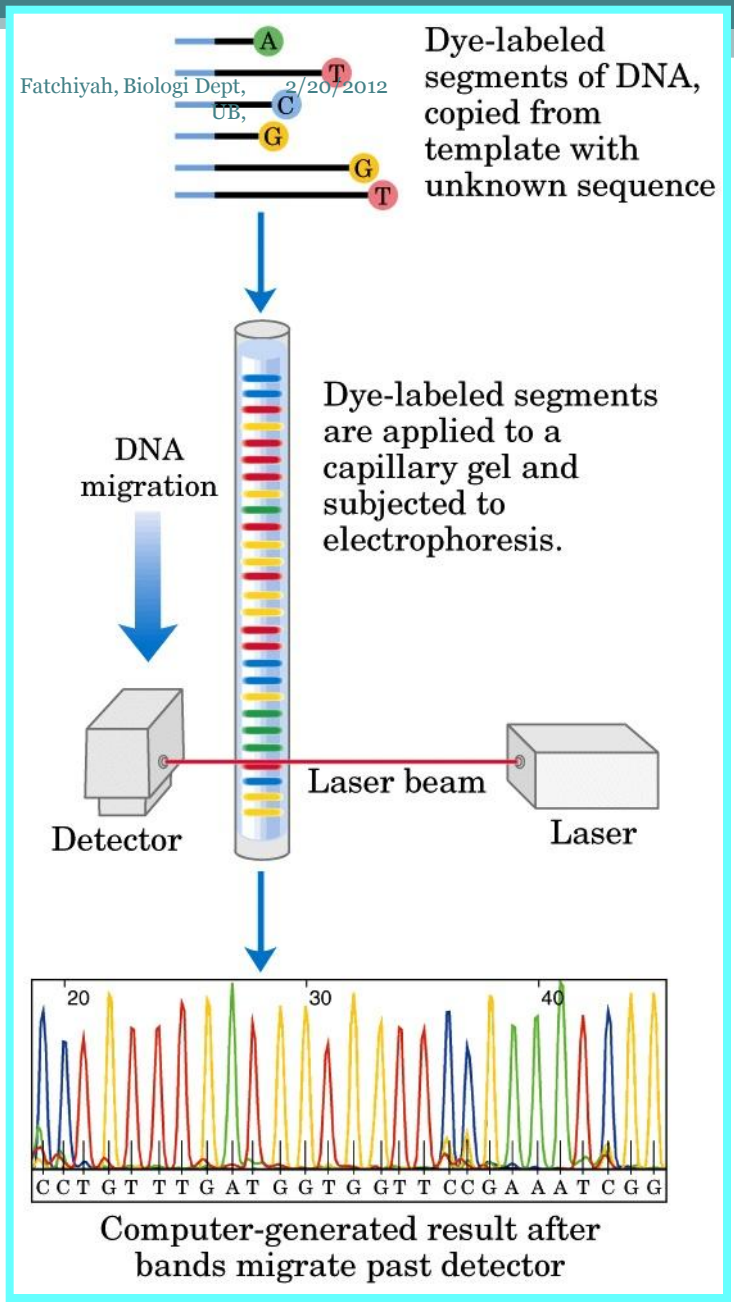
- Diagnosis
- Epidemiology
- Genetic engineering

DNA Sequencing

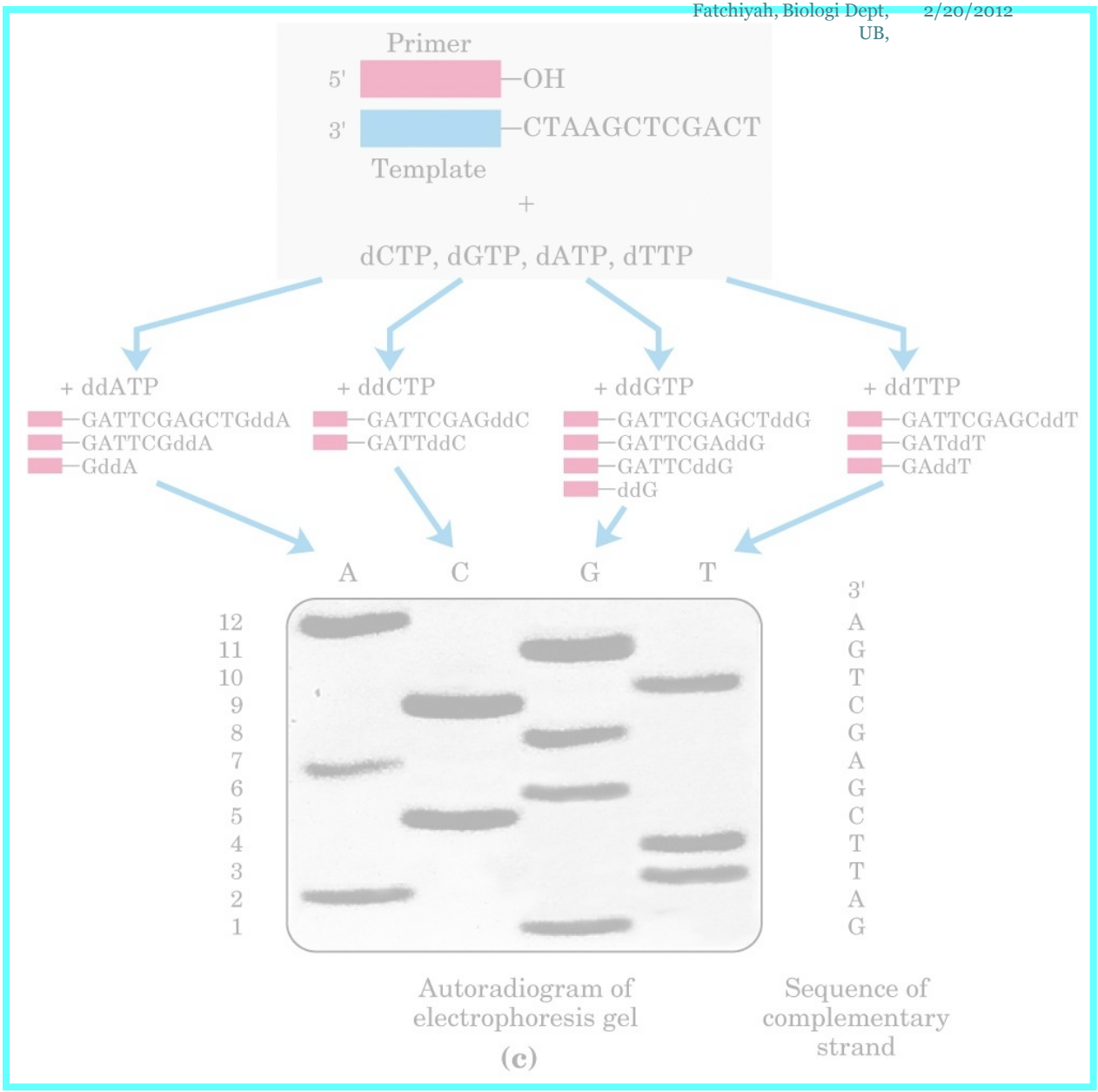




Dye-labeled segments of DNA, copied from template with unknown sequence



DNA Sequencing

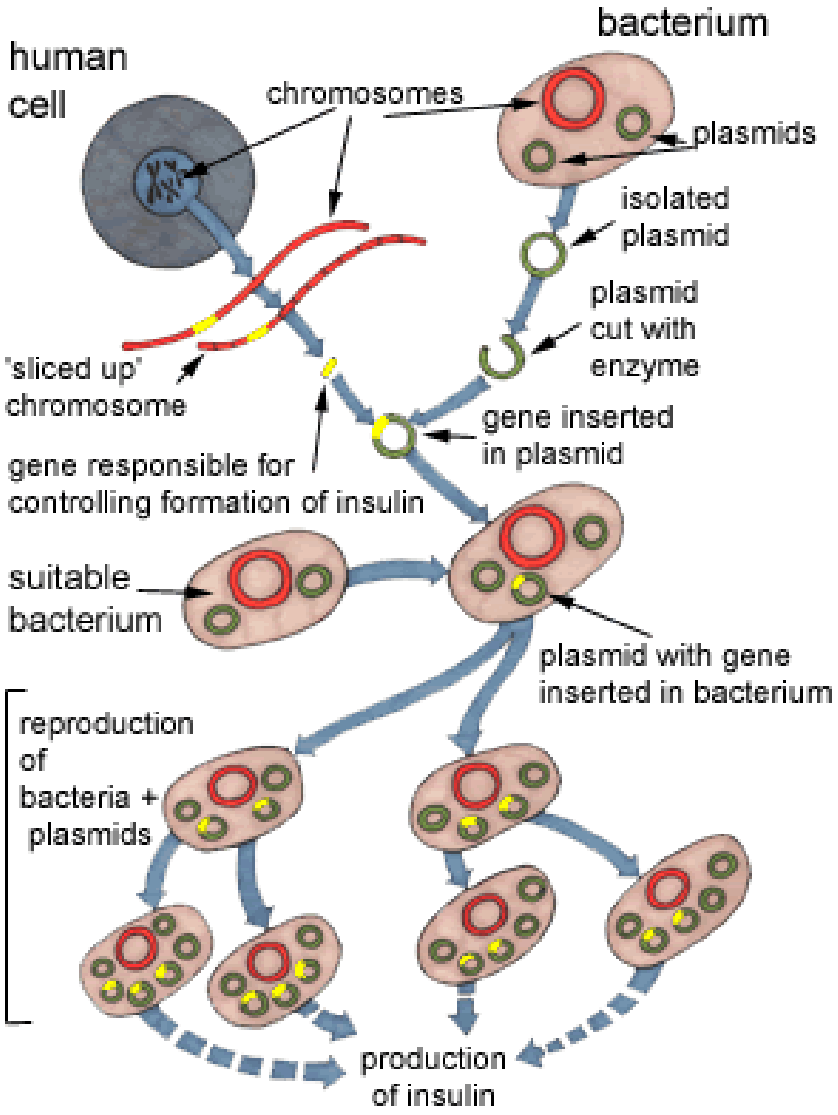


Genetic Engineering

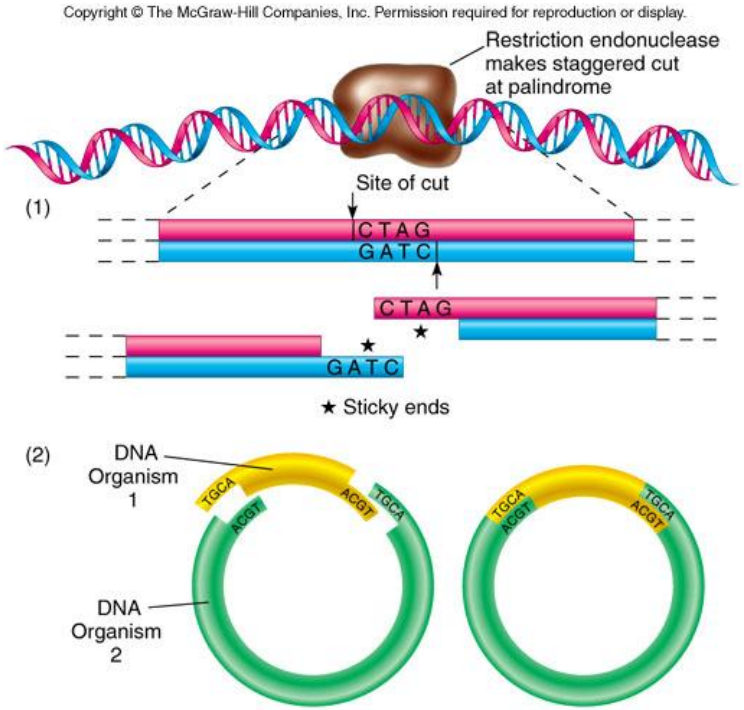
- Manipulating an organism's genome to
  - alter microbes, plants, and animals for our benefit
  - correct genetic defects in humans

Recombinant DNA

- Combining DNA from two different organisms



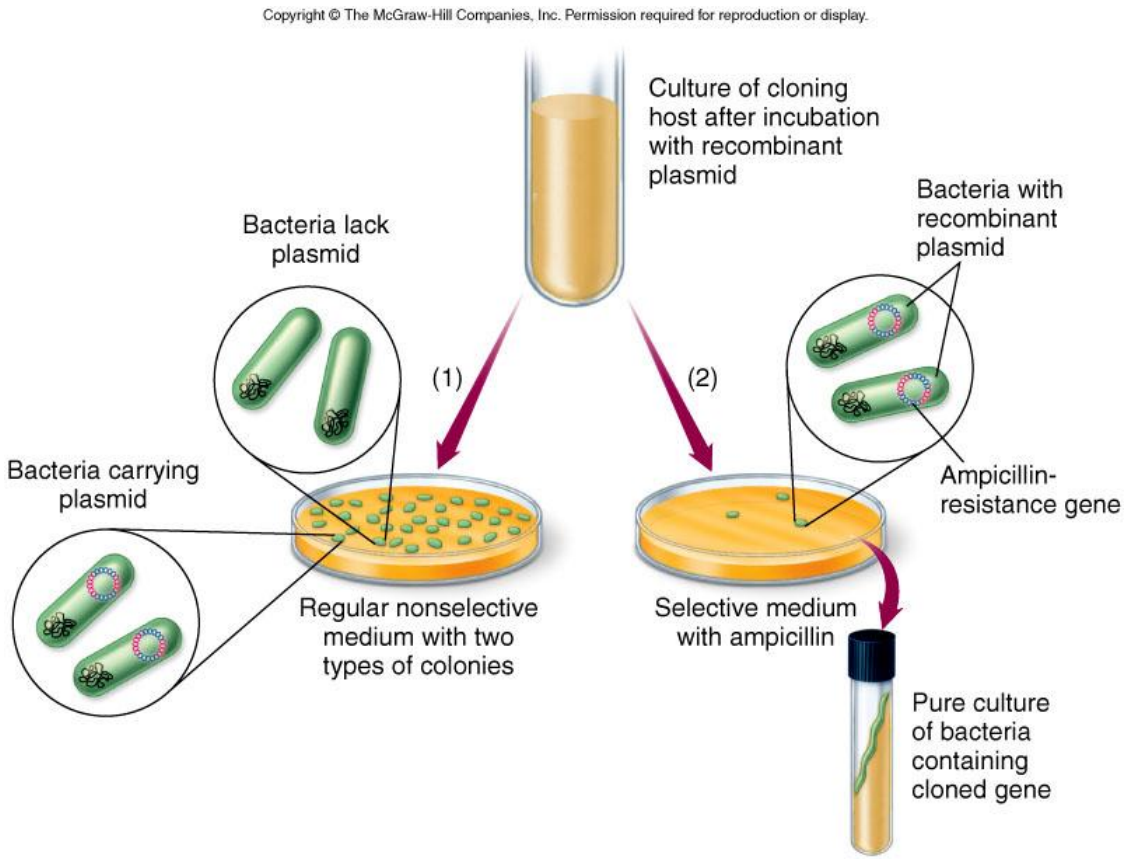
Useful Properties of DNA



(c) Action of restriction endonucleases. (1) A restriction endonuclease recognizes and cleaves DNA at the site of a specific palindromic sequence. Cleavage can produce staggered tails called sticky ends that accept complementary tails for gene splicing. (2) The sticky ends can be used to join DNA from different organisms by cutting it with the same restriction enzyme, ensuring that all fragments have complementary ends.

- Restriction endonucleases can cut DNA at specific sites, leaving sticky ends for insertion of new DNA

Selection of Altered Cells



- Antibiotic resistance gene used to identify recombinant cells

Genetically Modified Organisms

- Herbicide-resistant plants
- Bt cotton/corn (toxin gene from *Bacillus thuringiensis* that kills insects)
- Flavr-Savr tomatoes
- Golden rice (beta-carotene)
- Plant-based vaccines

A transgenic tomato plant

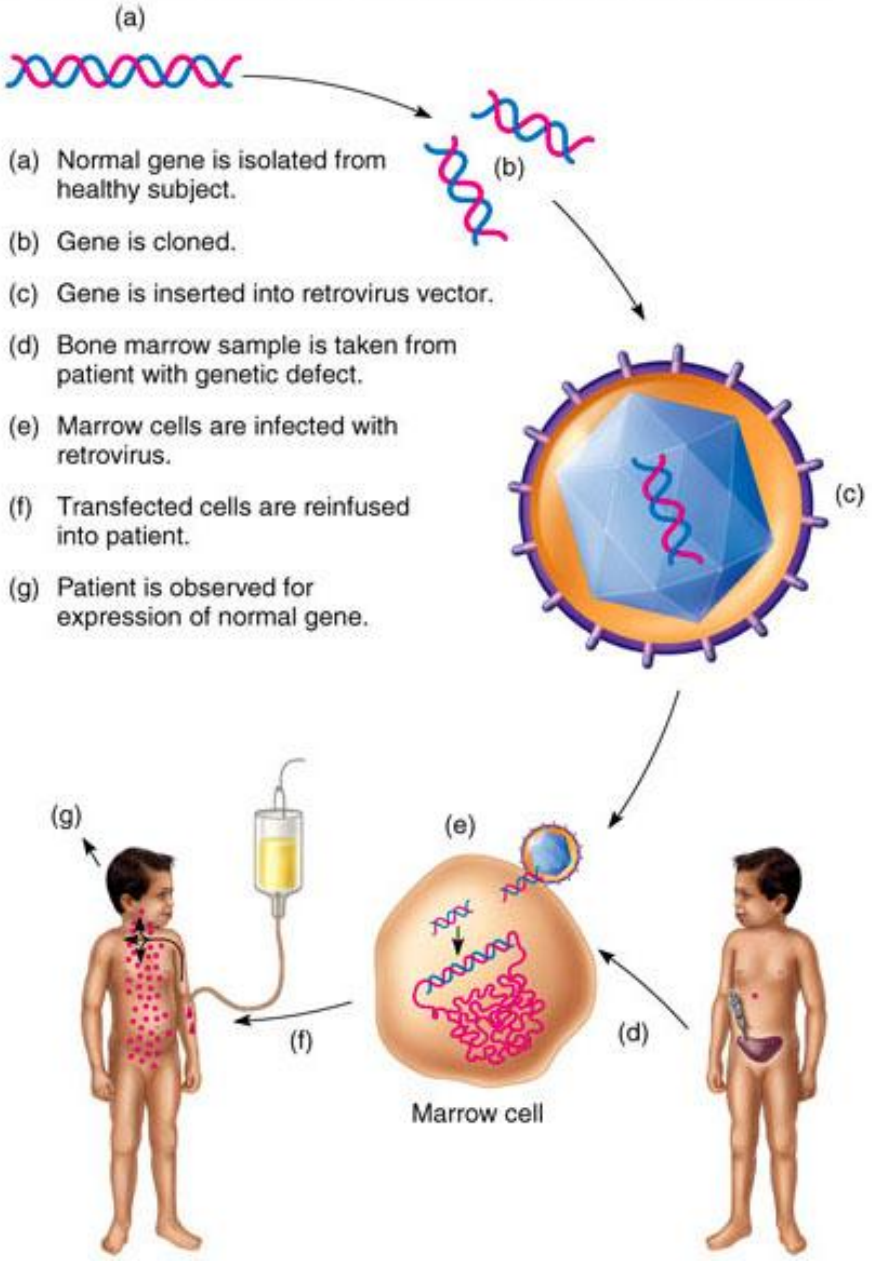
Fig. 13-18, p.349

Transgenic Animals

- “Knock-out” and transgenic mice: used to study immune system and genetic diseases
- Pigs: blood clotting Factor VIII, organs for transplantation
- Others: Human IL-2 (cancer), albumin (blood volume), growth hormone, tPA (dissolves clots)

- Two adult female ***Anopheles gambiae*** mosquitoes (ventral view).
- The one on the left is a mutant.
- Scientists are attempting to produce strains of these **mutant** mosquitoes, which are **unable to transmit malaria to humans**, in hopes that they will replace the malaria carriers

Fig. 13-14, p.342



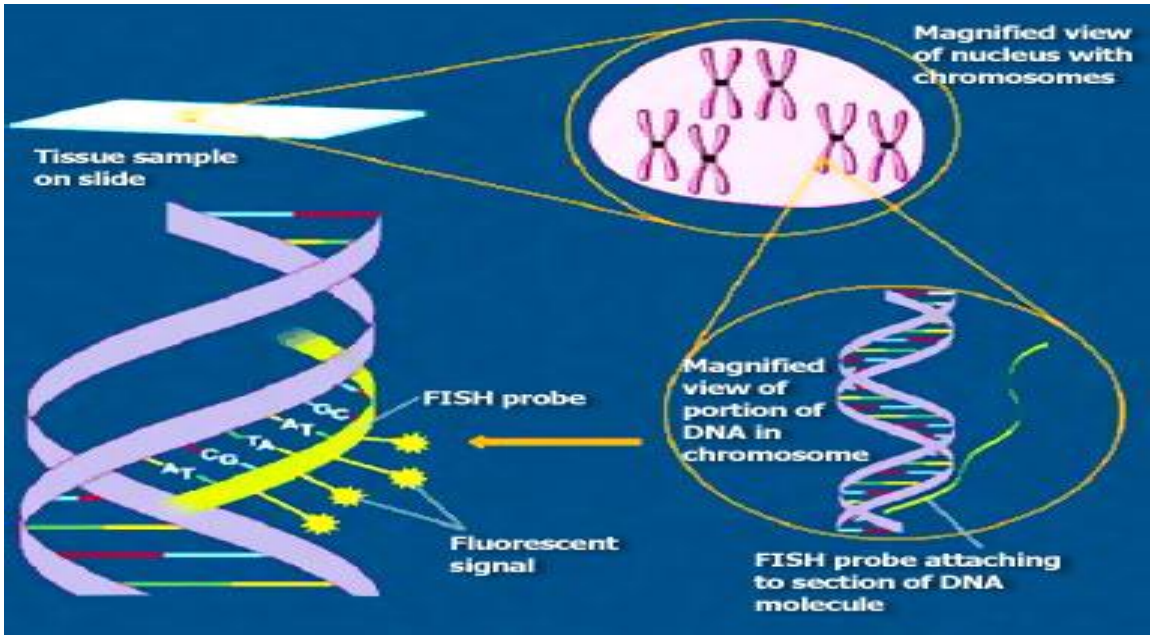
Genetically Engineering Humans

- Bone marrow supplies stem cells
- Successful replacement of gene for enzyme needed for lymphocyte development

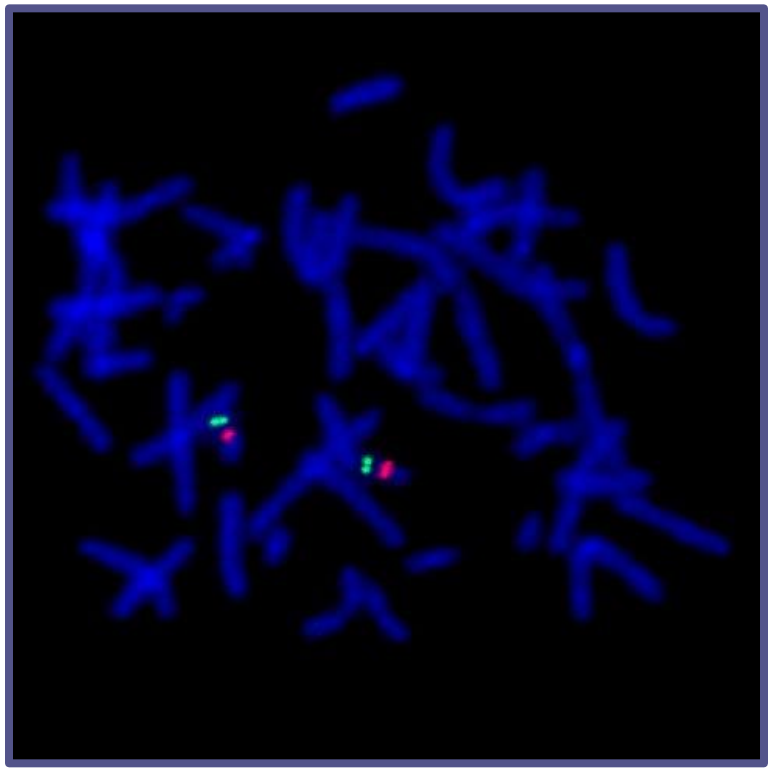
Difficulties in Genetically Engineering Humans

- Inserting gene in correct cells
- Inserting gene so it is expressed correctly
  - Orientation
  - Regulation
- Controlling virus vector
- Ethical issues

Fluorescent In-Situ Hibridization FISH



Metaphase FISH



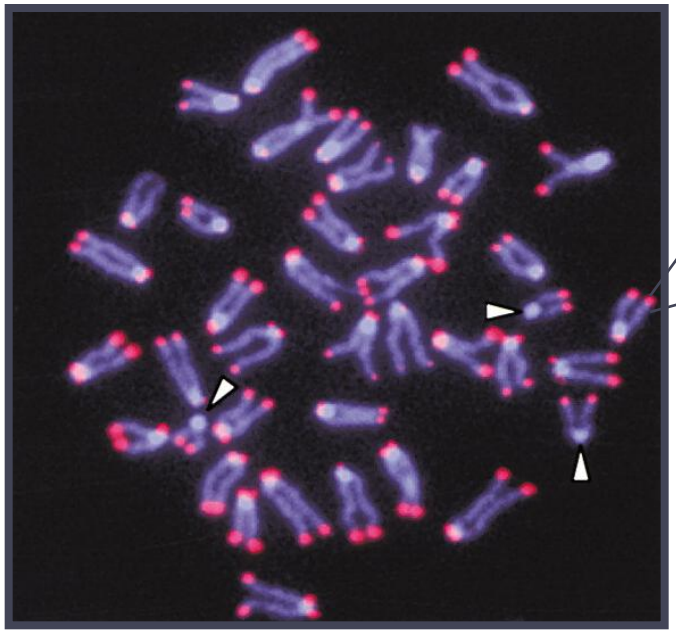
DNA Probe:

Green = Internal control

Red = DiGeorge region

Dual-color detection of ***DiGeorge/Velo-Cardio-Facial/CATCH 22/Shprintzen Syndrome*** which is caused by a microdeletion on chromosome 22. The green signal is an internal control. The red signal is located at the DiGeorge region at 22q11.2.

FISH of Telomeres



3' Telomeric overhang

Telomere repeats duplex

Telomeric sequence

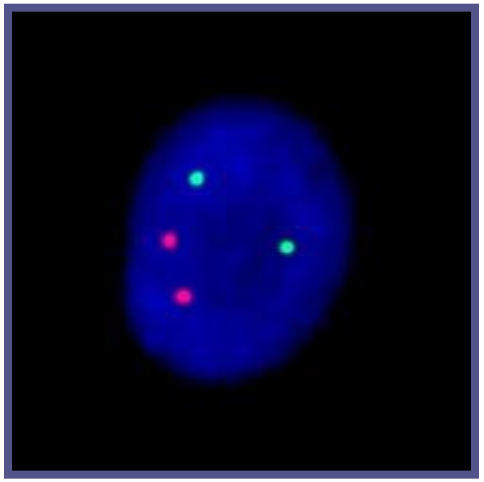
= TTAGGG in vertebrates

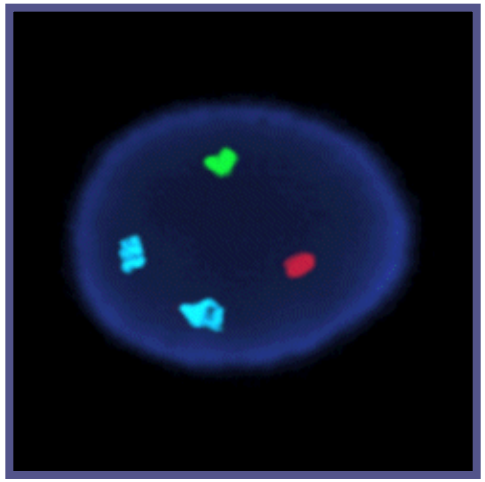
Metaphase Spread Stained by Q-FISH

Cell 2001, 107, 67-77

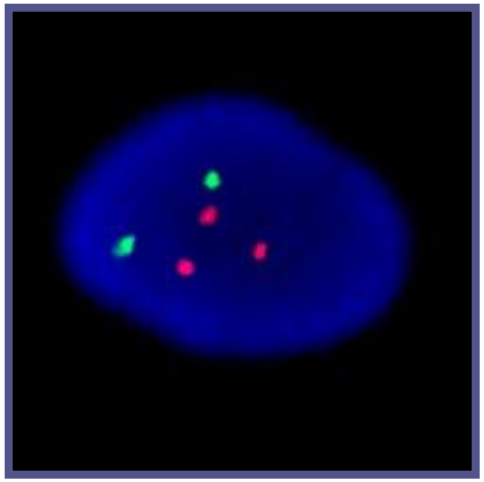
Aneuploid Screen Test Using Interphase FISH

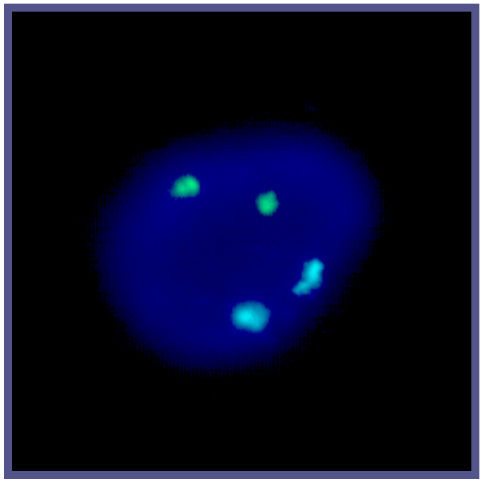
Normal male





Female fetus with trisomy-21





Probe:

Green 1 = Ch 13

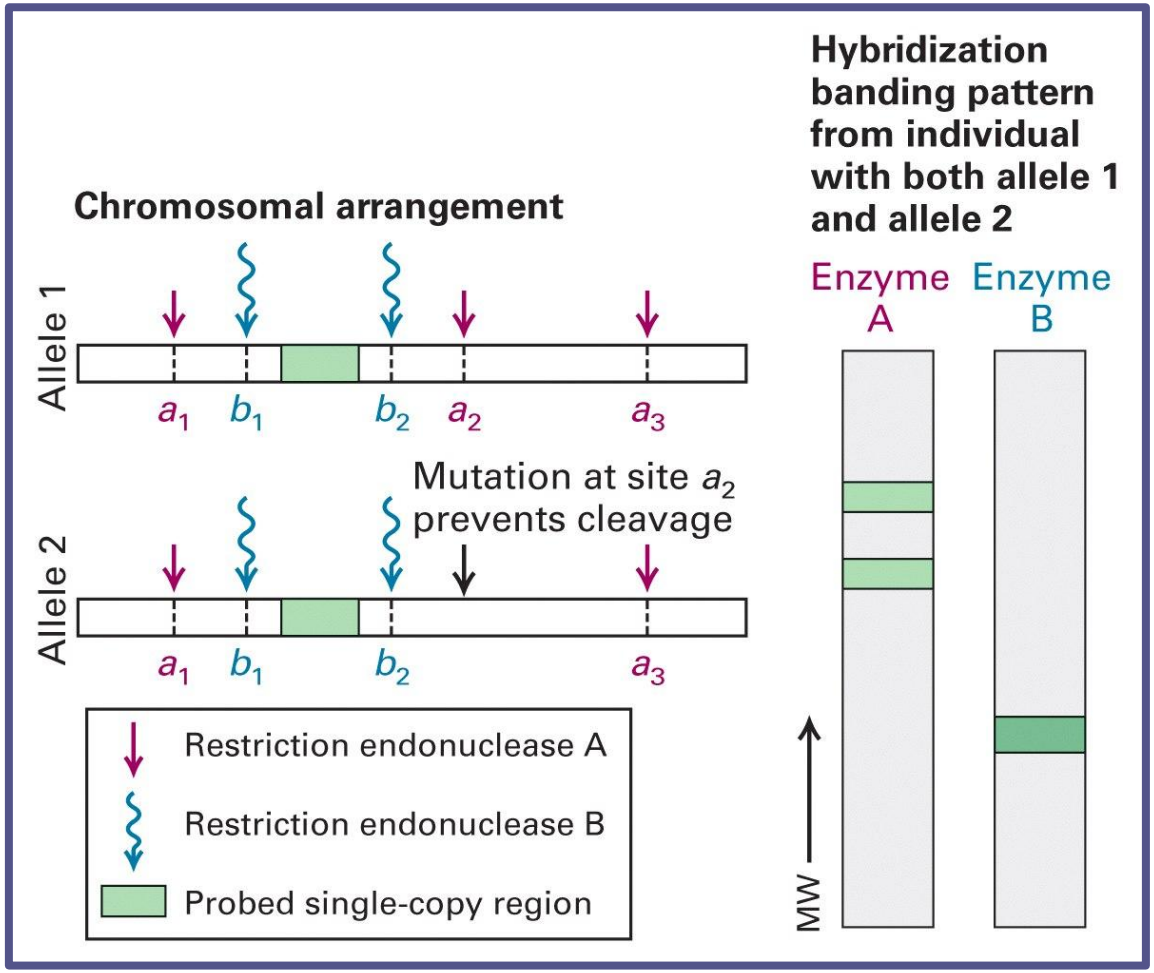
Red 1 = Ch 21

Aqua = Ch 18

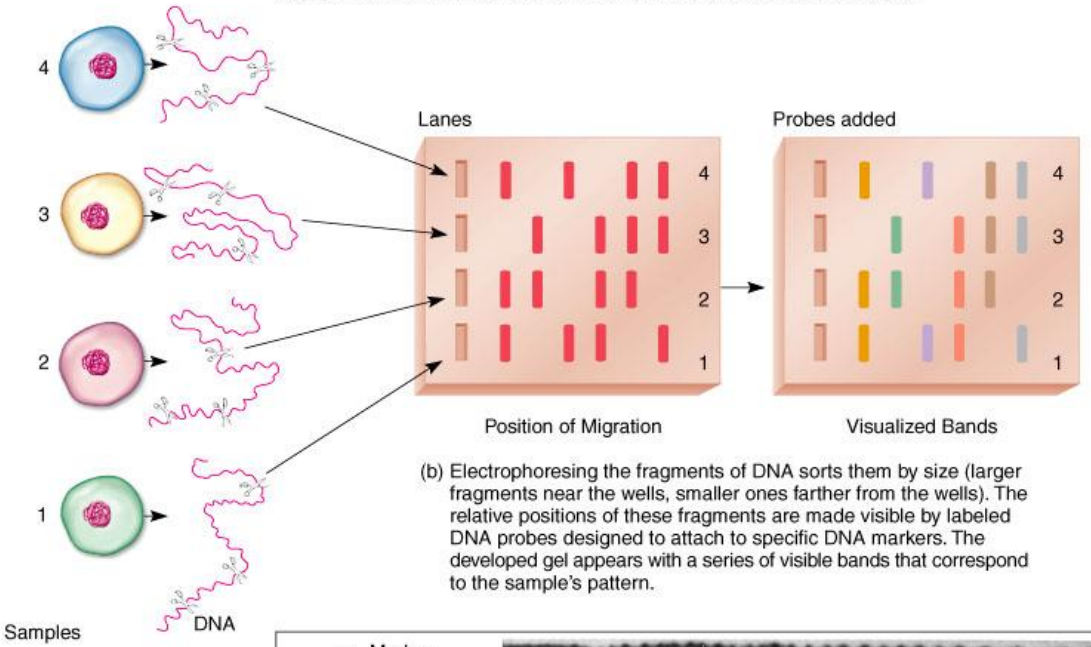
Green 2 = Ch X

Red 2 = Ch Y

Restriction Fragment Length Polymorphism (RFLP)

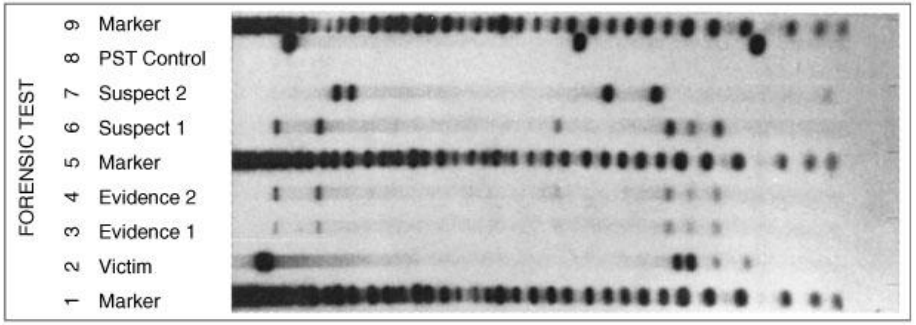


DNA Fingerprinting: Forensics



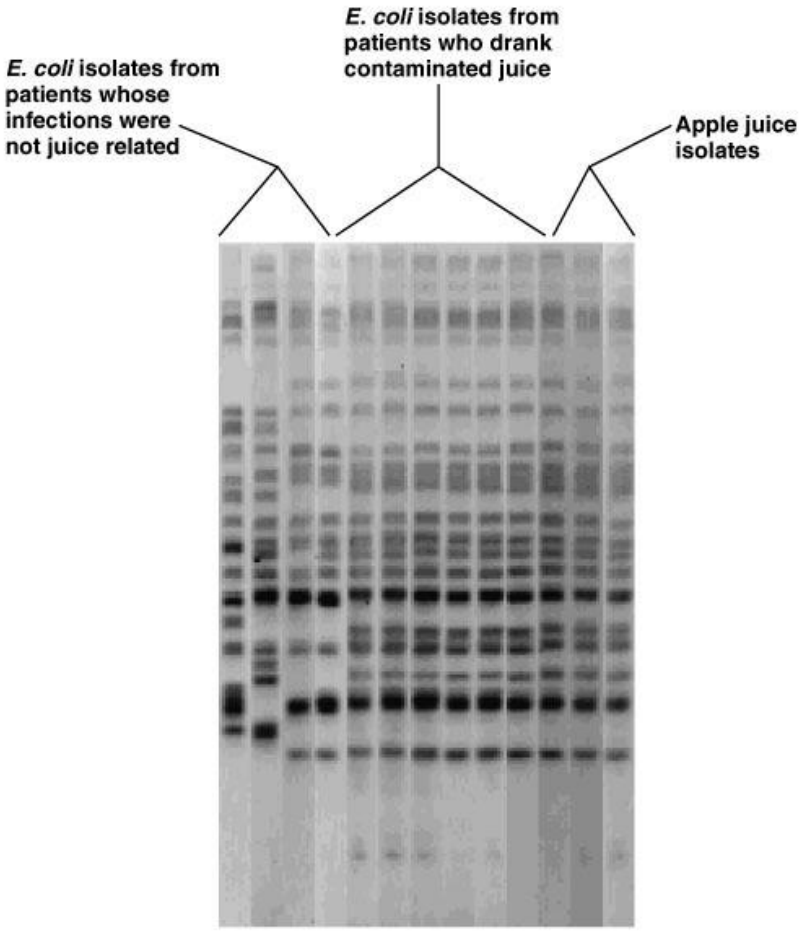
(a) Cells from different samples are processed to isolate their DNA. The DNA samples are exposed to endonucleases which snip them at specific sites into a series of different fragments.

(b) Electrophoresing the fragments of DNA sorts them by size (larger fragments near the wells, smaller ones farther from the wells). The relative positions of these fragments are made visible by labeled DNA probes designed to attach to specific DNA markers. The developed gel appears with a series of visible bands that correspond to the sample's pattern.



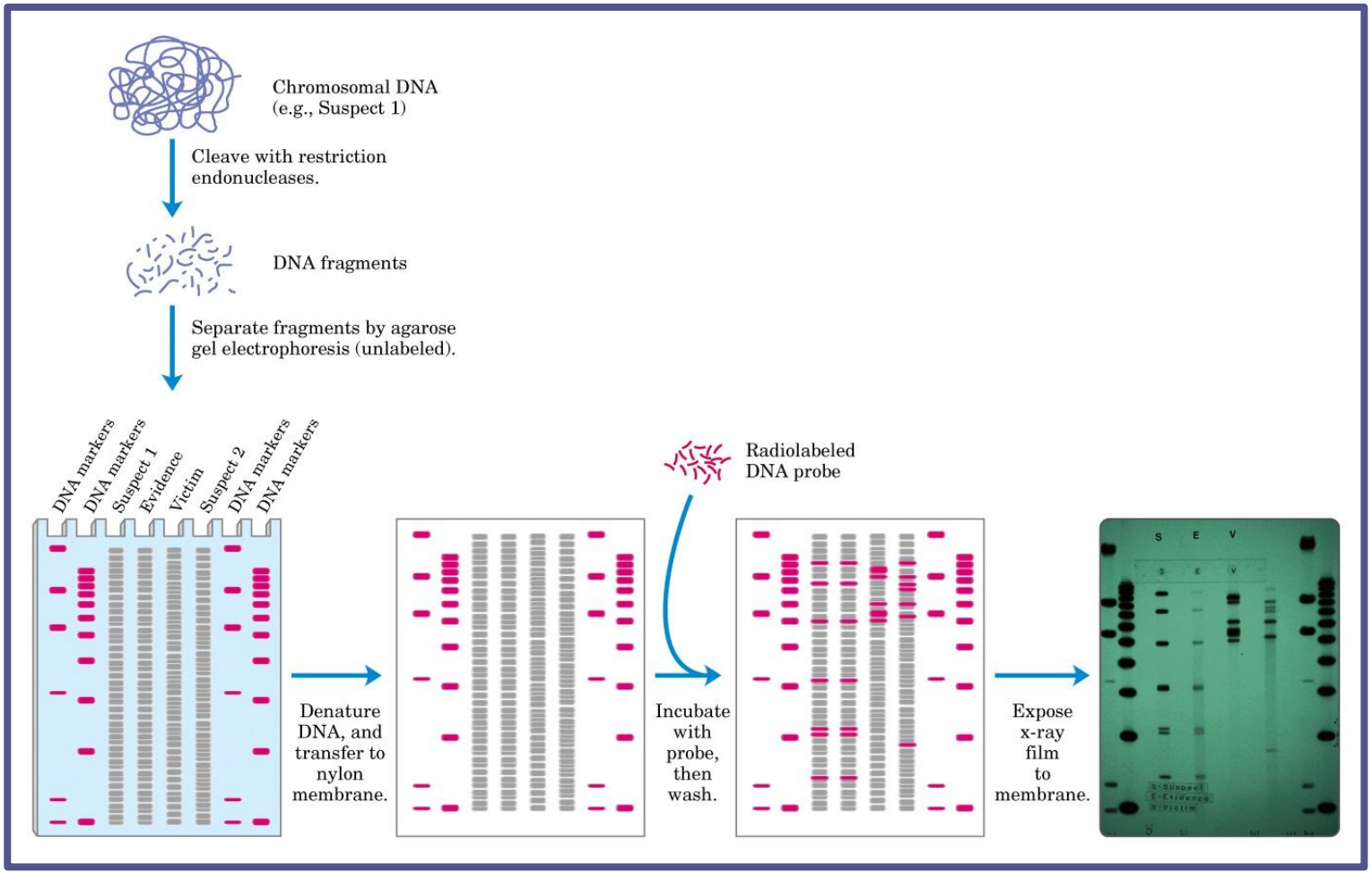
(c) An actual DNA fingerprint used in a rape trial. Control lanes with known markers are in lanes 1, 5, 8, and 9. The second lane contains a sample of DNA from the victim's blood. Evidence samples 1 and 2 (lanes 3 and 4) contain semen samples taken from the victim. Suspects 1 and 2 (lanes 6 and 7) were tested. Can you tell by comparing evidence and suspect lanes which individual committed the rape?

DNA Fingerprinting: Epidemiology

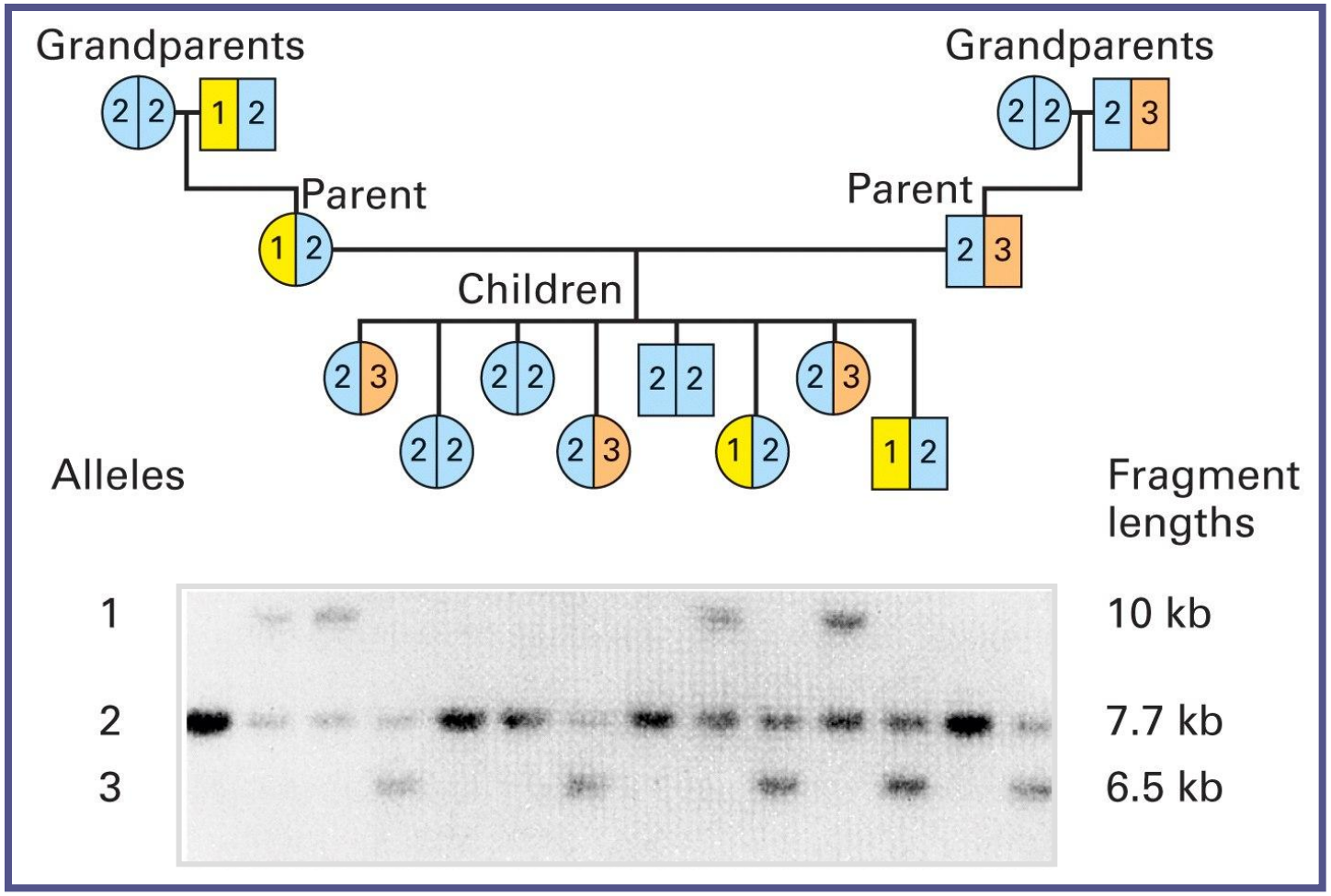


- Comparison of DNA from
  - patients
  - food

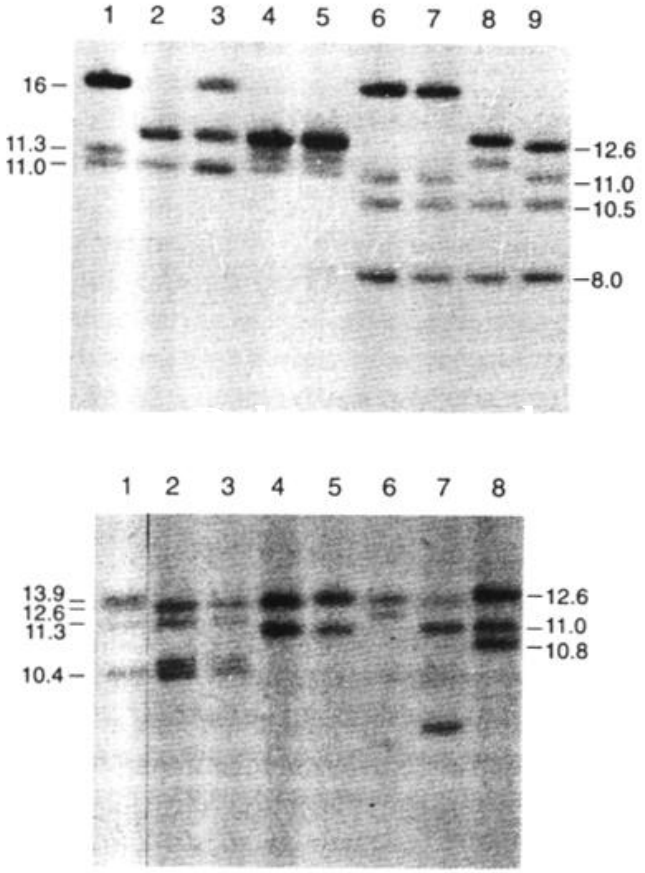
Southern Hybridization

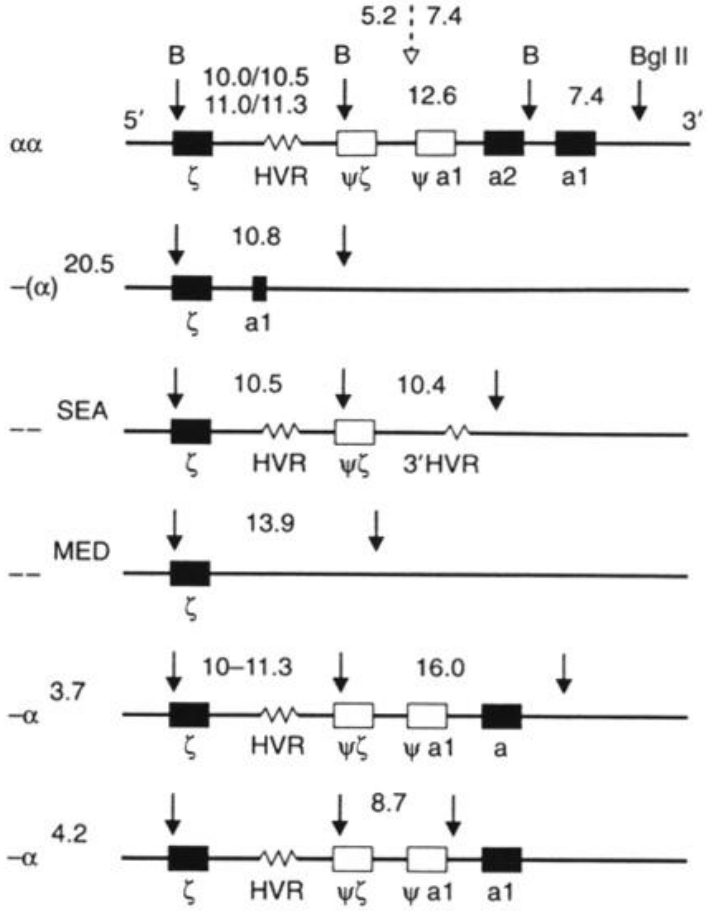


RFLP as Genetic Marker



RFLP and Southern Blot Analysis





Allele	*Bgl* II/ζ
αα	12.6 or 5.2 10–11.3
$-\alpha^{3.7}$	16 10–11.3
$-\alpha^{4.2}$	10–11.3 8.0
$-(\alpha)^{20.5}$	10.8
– –MED	13.9
– –SEA	10.5
– –SA	7.0
– –BRIT	7.5
– –THAI	None
– –FIL	None

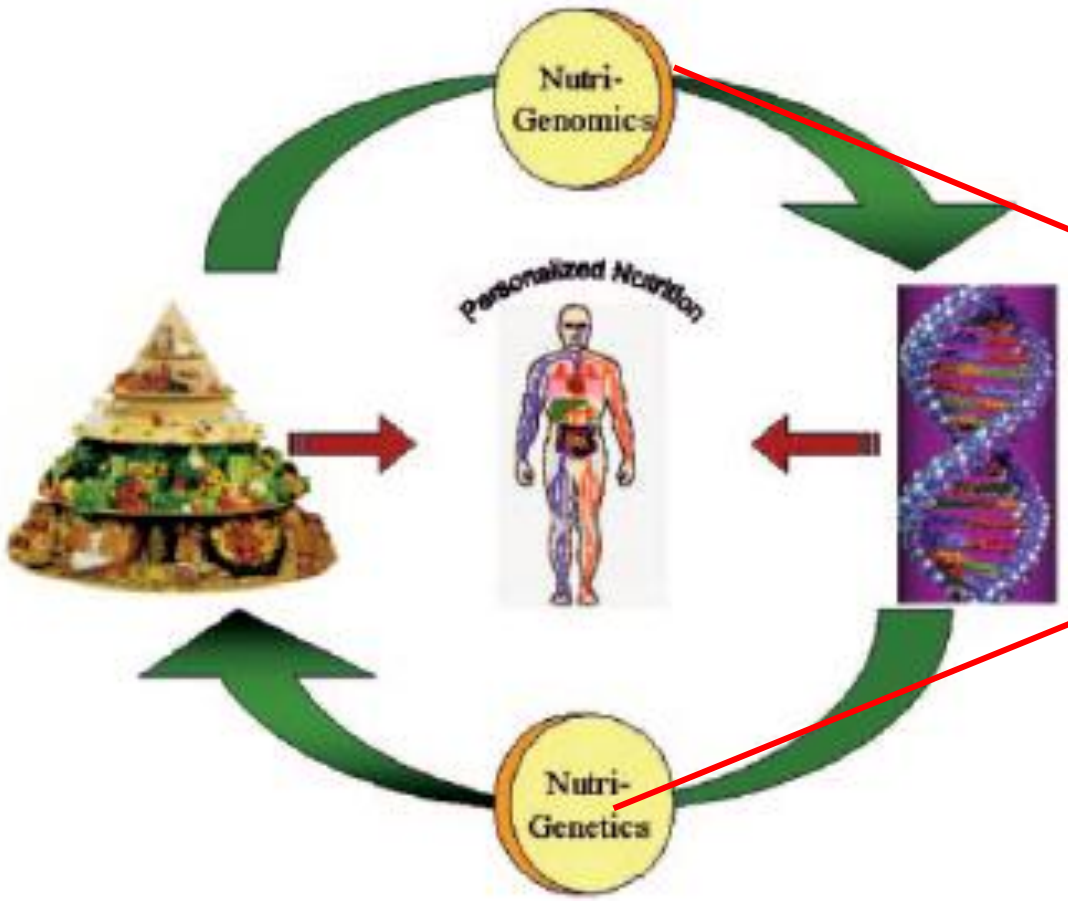
Nutrigenomics

- The study of how different foods can interact with particular genes to increase the risk of diseases such as type 2 diabetes, obesity, heart disease and some cancers

- Goal: Use of personalized diets to prevent or delay the onset of disease and optimize and maintain human health

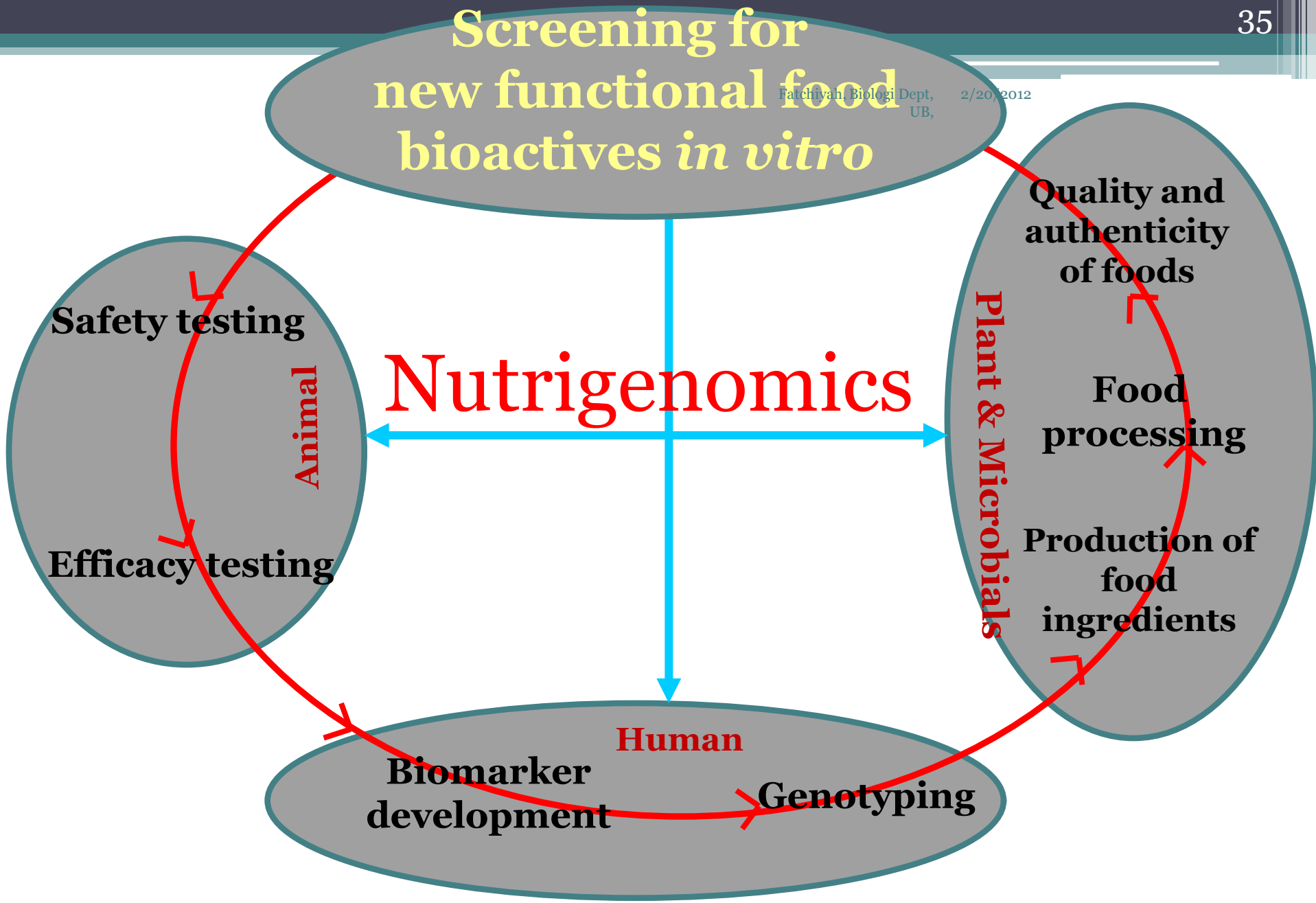
http://nutrigenomics.ucdavis.edu/pressarticles.htm

Nutrigenomics and nutrigenetics: two sides of a coin

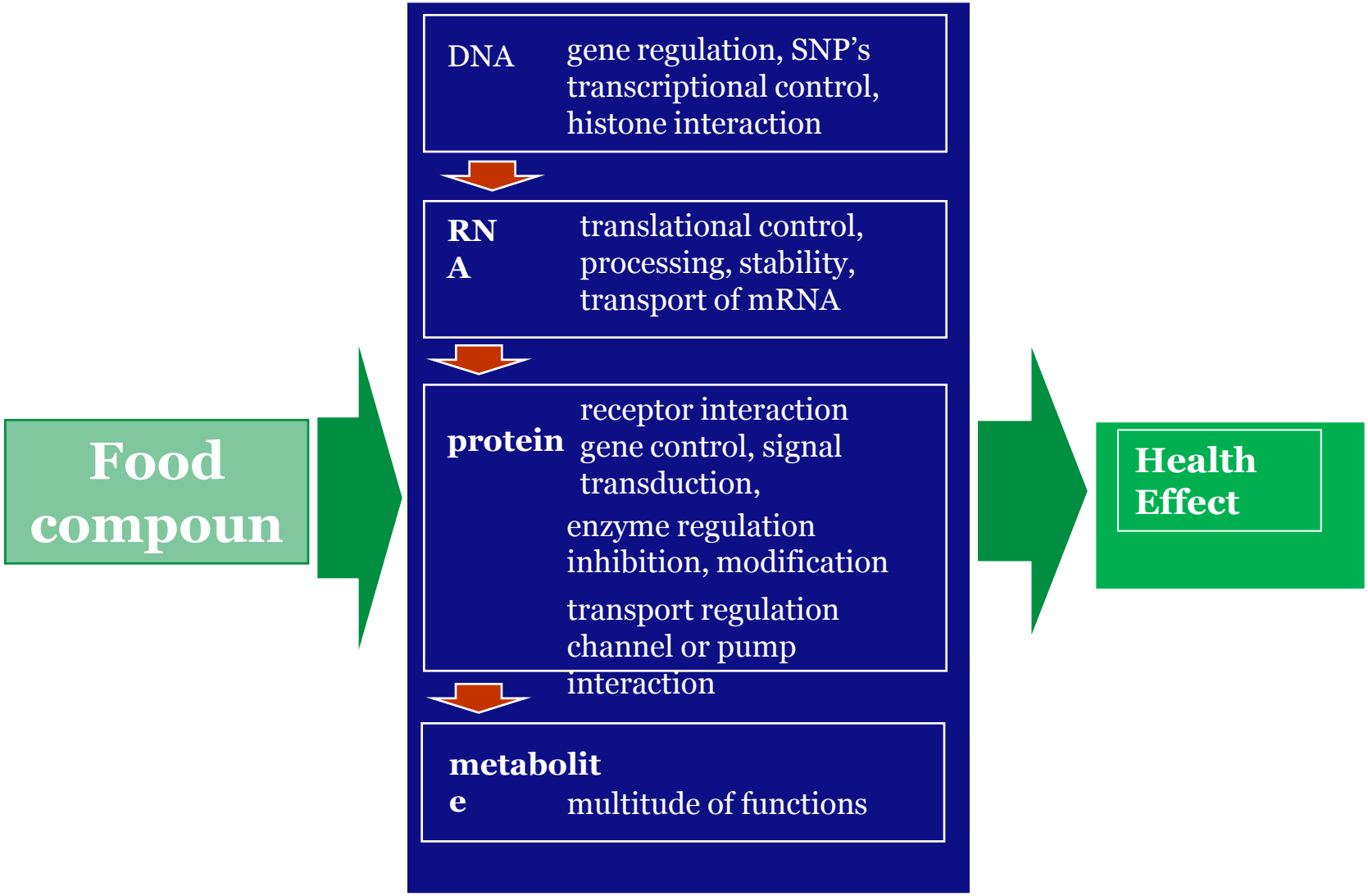


- For personalized nutrition:
  - effects of diet on body-metabolism
  - influence of genotype on nutritionally related diseases

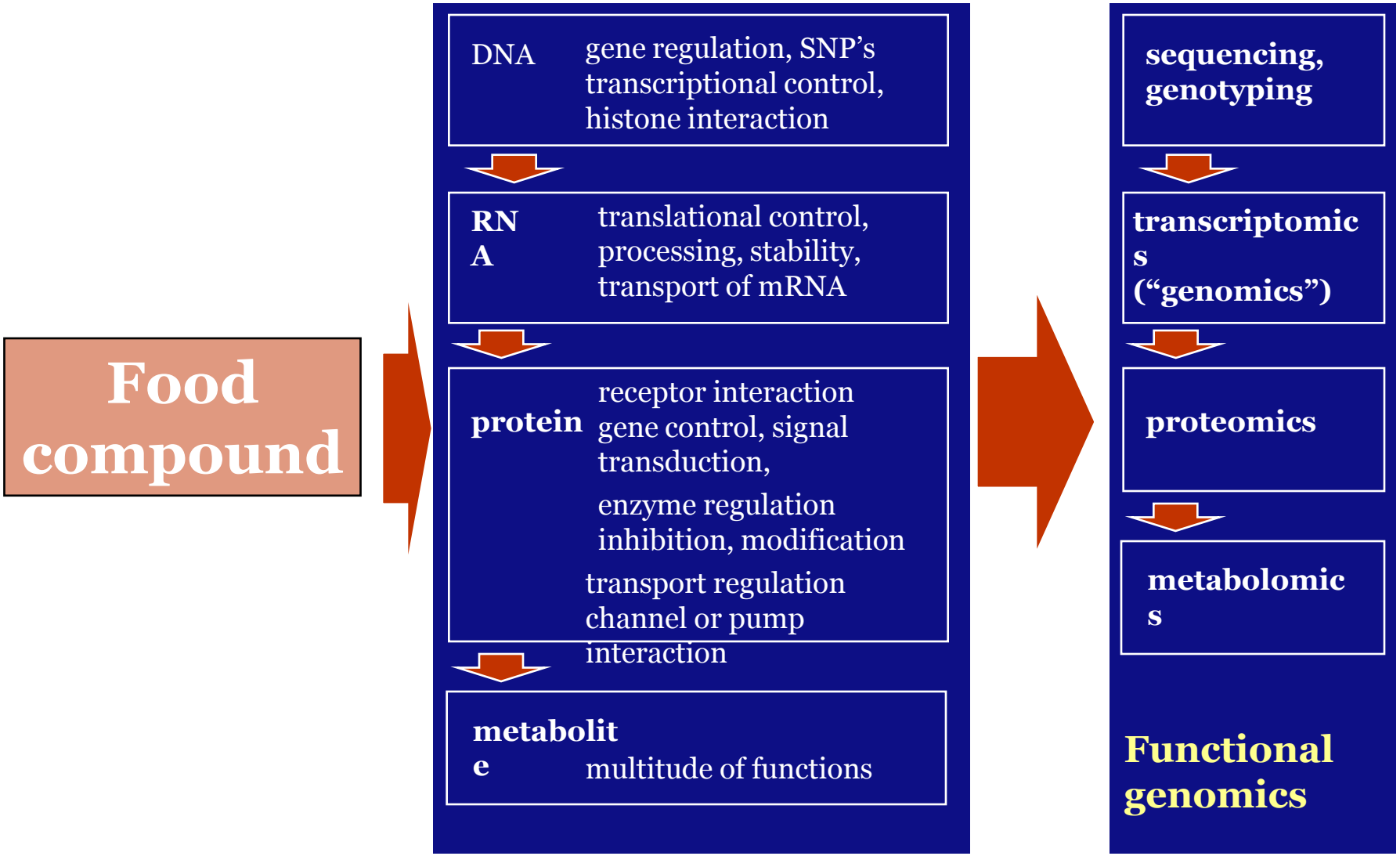
Mutch, FASEB 2005


Screening for new functional food bioactives *in vitro*
Nutrigenomics
Safety testing
Animal
Efficacy testing
Quality and authenticity of foods
Plant & Microbials
Food processing
Production of food ingredients
Human
Biomarker development
Genotyping

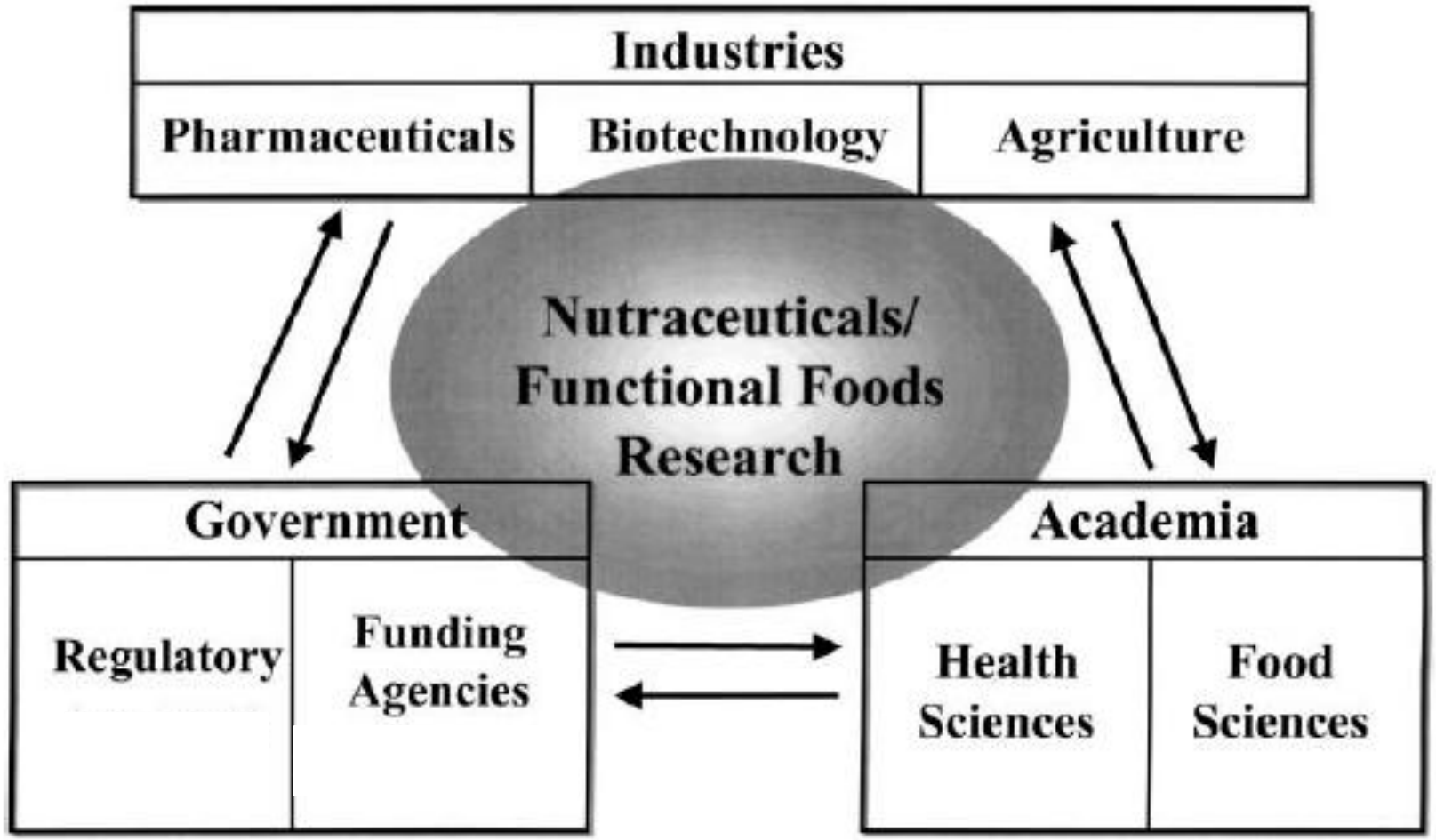
Health effects of food compounds mostly are related to specific interactions on molecular level



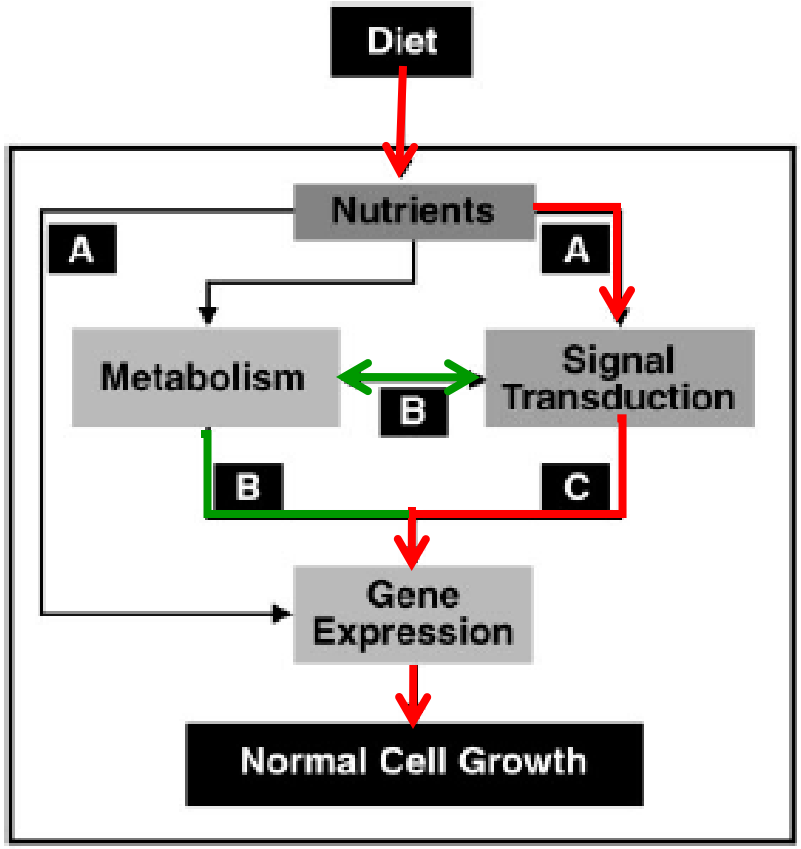
Health effects of food compounds mostly are related to specific interactions on molecular level



Interrelated strategies for research on nutraceuticals and functional foods



Fate and activities of nutrients in the cell



Kaput J, Physiol Genomics 2004

May be involved in gene regulation or cell-signaling

Thank you