Novel Applications of Genetic Engineering / Recombinant DNA Technology

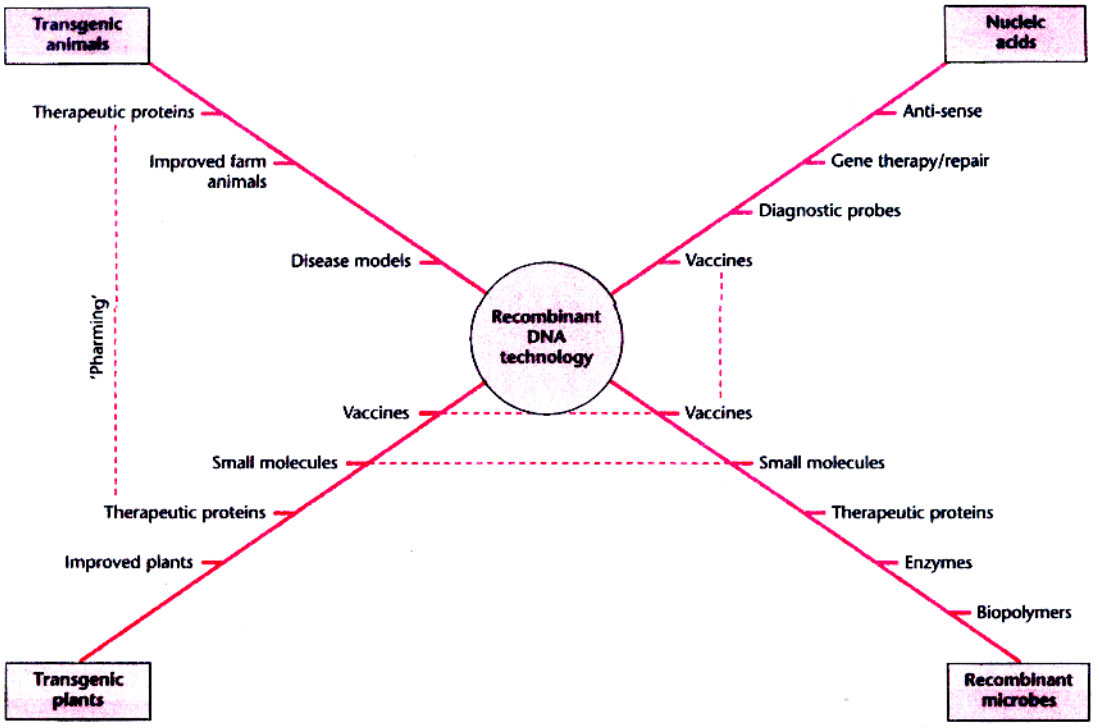


Fig. 14.1 The different ways that recombinant DNA technology has been exploited.

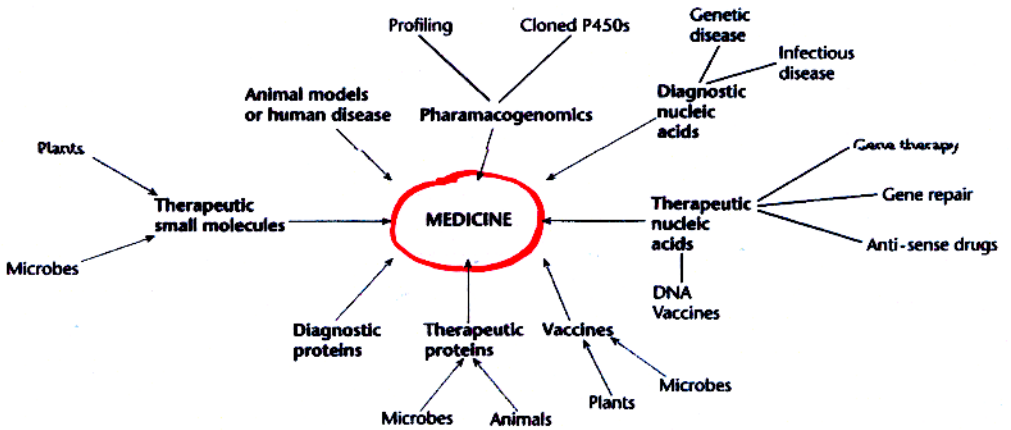


Fig. 1.1 The impact of gene manipulation on the practice of medicine.

For Example!

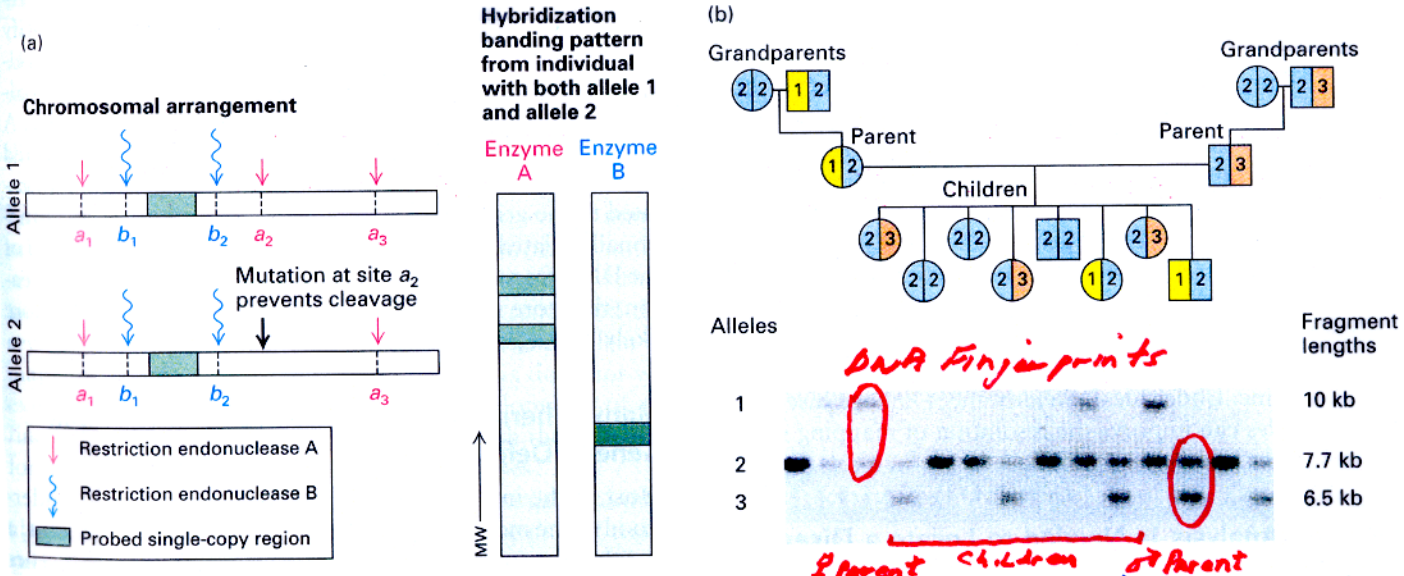
Genetic Engineering Has Lead to the Ability to Test For Disease Genes

TABLE 9-3 Common Inherited Human Diseases

Disease	Molecular and Cellular Defect	Incidence
AUTOSOMAL RECESSIVE		
Sickle-cell anemia	Abnormal hemoglobin causes deformation of red blood cells, which can become lodged in capillaries; also confers resistance to malaria.	1/625 of sub-Saharan African origin
Cystic fibrosis	Defective chloride channel (CFTR) in epithelial cells leads to excessive mucus in lungs.	1/2500 of European origin
Phenylketonuria (PKU)	Defective enzyme in phenylalanine metabolism (tyrosine hydroxylase) results in excess phenylalanine, leading to mental retardation, unless restricted by diet.	1/10,000 of European origin
Tay-Sachs disease	Defective hexosaminidase enzyme leads to accumulation of excess sphingolipids in the lysosomes of neurons, impairing neural development.	1/1000 Eastern European Jews
AUTOSOMAL DOMINANT		
Huntington's disease	Defective neural protein (huntingtin) may assemble into aggregates causing damage to neural tissue.	1/10,000 of European origin
Hypercholesterolemia	Defective LDL receptor leads to excessive cholesterol in blood and early heart attacks.	1/122 French Canadians
X-LINKED RECESSIVE		
Duchenne muscular dystrophy (DMD)	Defective cytoskeletal protein dystrophin leads to impaired muscle function.	1/3500 males
Hemophilia A	Defective blood clotting factor VIII leads to uncontrolled bleeding.	1–2/10,000 males

DNA Testing Allows Genes to be TRACED FROM Generation to Generation

and has raised many new societal issues & challenges — such as?



EXPERIMENTAL FIGURE 9-46 Restriction fragment length polymorphisms (RFLPs) can be followed like genetic markers. (a) In the example shown, DNA from an individual is treated with two different restriction enzymes (A and B), which cut DNA at different sequences (*a* and *b*). The resulting fragments are subjected to Southern blot analysis (see Figure 9-26) with a radioactive probe that binds to the indicated DNA region (green) to detect the fragments. Since no differences between the two homologous chromosomes occur in the sequences recognized by the B enzyme, only one fragment is recognized by the probe, as indicated by a single hybridization band. However, treatment with enzyme A produces fragments of two different lengths (two bands are seen), indicating that a mutation has caused the loss of one of the *a* sites in one of the two chromosomes. (b) Pedigree based on RFLP analysis of the DNA from a region known to be present on chromosome 5. The DNA samples were cut with the restriction enzyme *Taq*I and analyzed by Southern blotting. In this family, this region of the genome exists in three allelic forms characterized by *Taq*I sites spaced 10, 7.7, or 6.5 kb apart. Each individual has two alleles; some contain allele 2 (7.7 kb) on both chromosomes, and others are heterozygous at this site. Circles indicate females; squares indicate males. The gel lanes are aligned below the corresponding subjects. [After H. Donis-Kelier et al., 1987, *Cell* **51**:319.]

in one or both parents!!

The HC70A Class will be DNA fingerprinted this Quarter!!

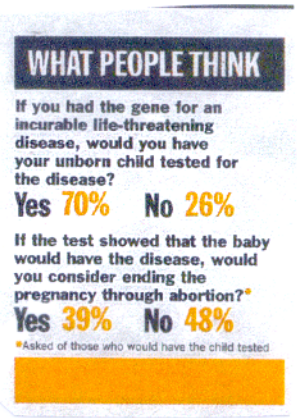
in other organisms too!

Genetic Engineering Technology Has Led to Many Legal & Ethical issues

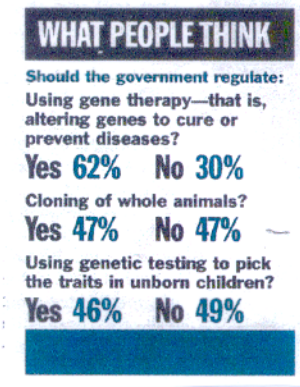
1. Patenting Living organisms, Cells, & Genes
2. Regulating "Experimentation" — recombinant DNA, stem cells, transgenic plants and animals
3. Regulating Release of genetically modified organisms into environment — crops, farm animals, mosquitos
4. Genetic Testing — genetic data bases, voluntary, involuntary, newborn screening, criminals, suspects
5. Genetic Discrimination — insurance, workplace, society
6. Eugenics — Genetic Enhancement —
7. Reproductive Rights — genetic enhance child, wrongful birth suits
8. Gene therapy — correcting genetic disorders
9. Gene Testing Companies — Liabilities
10. Human Cloning — Reproductive Rights — Regulate?
11. Synthetic Genomes — What is life?

Issues that need to be Resolved By Informed Public Choices

Genetic Testing

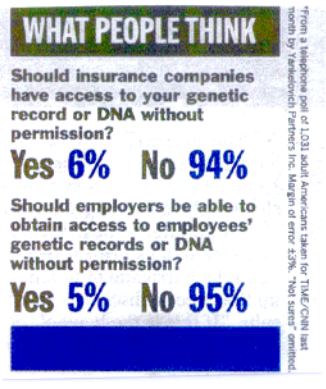


Gene Therapy



Genetic Privacy

Genetic Discrimination



Genetic Databases

Genetically Engineered Food

and need to be guided by sound science!!

Issues Raised By Genetic Engineering Technology – Like all new technologies society & people are affected

Science-philosophy arguments concerning genetic engineering

categorical argument

Some human activities such as genetic engineering are fundamentally reprehensible. Developing this technology, "man plays God" and claims competencies beyond his capacities, degrading nature to the course of his technical manipulations.

pragmatic argument

The key objective of genetic engineering is to reduce the suffering of diseased individuals. The procedures which are applied must, however, be safe, and the patient must be able to decide if he or she wishes to apply genetic diagnosis or therapy.

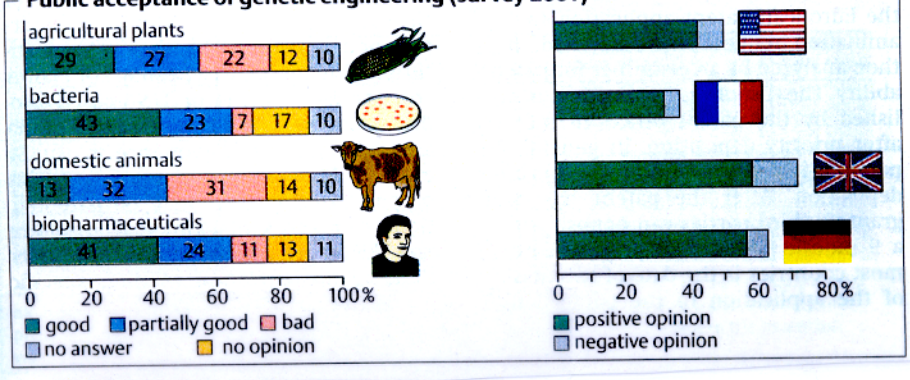
social policy argument

The social effects of genetic engineering cannot be estimated. In genetic therapy, wrong priorities are chosen, better prophylaxis would be more desirable. We start down a slippery slope that will lead us involuntarily to inhumane practices towards the next generations ("eugenics bottom up")

Problematic areas of genetic research

topic	state of the art	regulation or trend
cloning of humans	cloning of animals possible	not permitted
use of embryonic stem cells	growing expertise	permitted, but regulated
artificial insemination, sexing, surrogate mothers	state of the art in animals	artificial insemination permitted, sexing and surrogate mothers forbidden
prenatal diagnosis	cytological methods established, DNA-based diagnosis partially established	permitted, abortion permitted after medical indication
identifying genetic risks by genetic screening	possible for some monogenic diseases	under debate if one gene defect is predictive and if diagnosis is acceptable for incurable diseases; strict data protection required towards employers, insurance companies
knockout animals for drug research	widely established	generally accepted, but hotly debated by animal protection groups
food and biopharmaceutical production using transgenic animals or plants	many techniques established	debated in view of consumer protection, animal protection, ecological consequences
transgenic microorganisms or cell lines for production of biopharmaceuticals	established	widely accepted

Public acceptance of genetic engineering (survey 2001)



Why it is IMPORTANT TO UNDERSTAND the SCIENCE BEHIND Genetic Engineering!!!!!

That's what this CLASS is About! 26

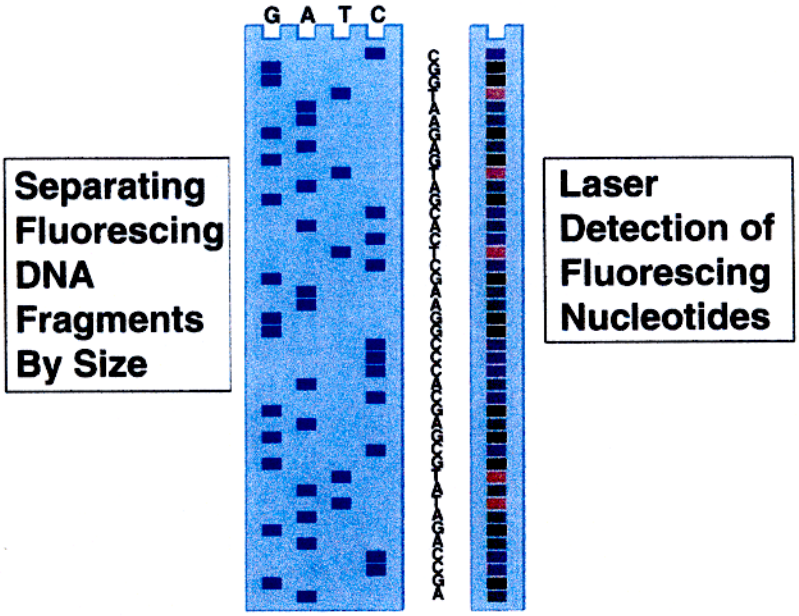
We Live in the Genomics Era – The Age of the Genome !!

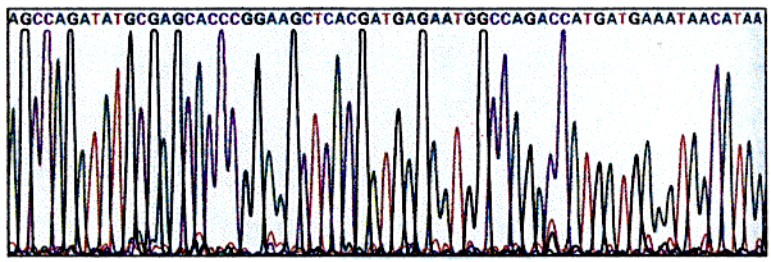
Genetic Engineering GAVE "Birth" to this ERA!!

IT IS POSSIBLE TO ISOLATE AND SEQUENCE Every Gene in A Genome!

Need to Clone DNA Segment First

Genome Sequencing Using Computers and Robotics





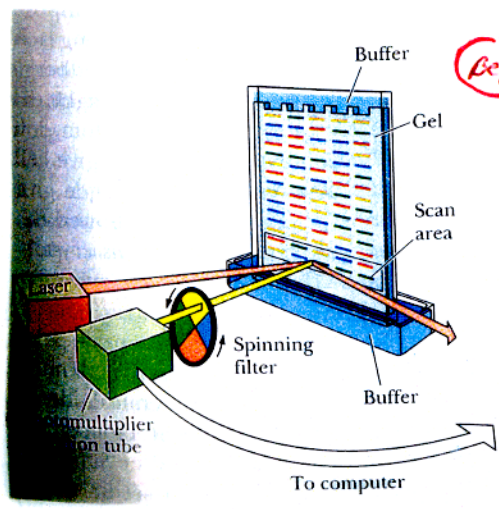
Computer Visualization of DNA Sequence

Gene Chemical Sequence

Begin → End

CCGGAAGCATAAAGTGTACAT

1. What Makes gene unique
2. What it does!
3. How it works in cell!



Specific Order → Specific function!! in cell

The Genomes of all major CLASSES of ORGANISMS Have Been Sequenced
Including HUMANS!

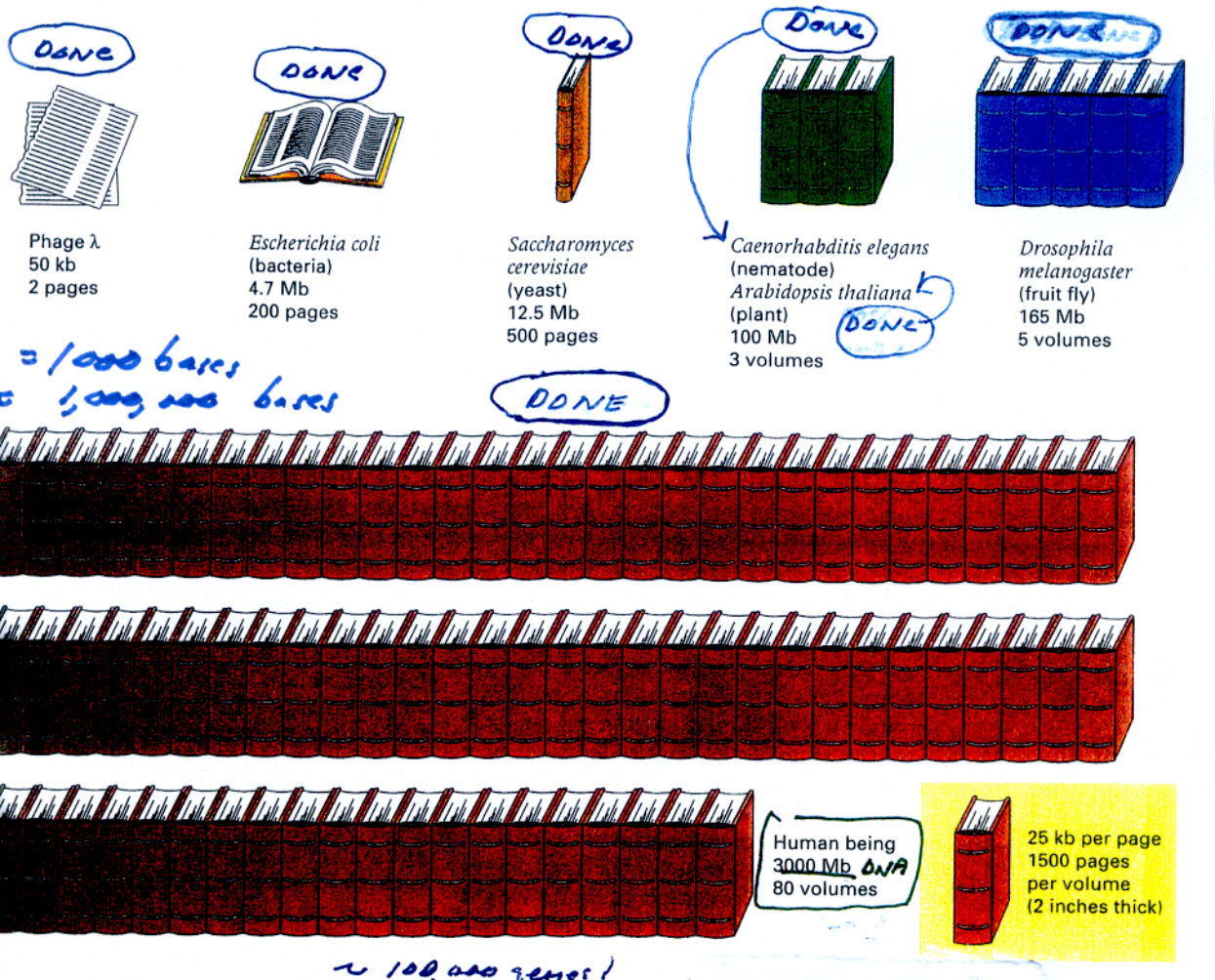


Figure 9.21
Relative sizes of genomes if they were printed at 25,000 characters per page and bound in 1500-page volumes. One volume would contain about as many characters as a telephone book 2.5 inches thick. The *E. coli* genome would require about 200 pages, yeast 500 pages, and so forth.

MOUSE Both done!!
pufferfish

By 2010 (or sooner) all of the genes of each major group of organisms on Earth will have been isolated, sequenced & their functions revealed!

ALL genes in these organisms have been identified – e.g., Mouse & Humans have same genes!

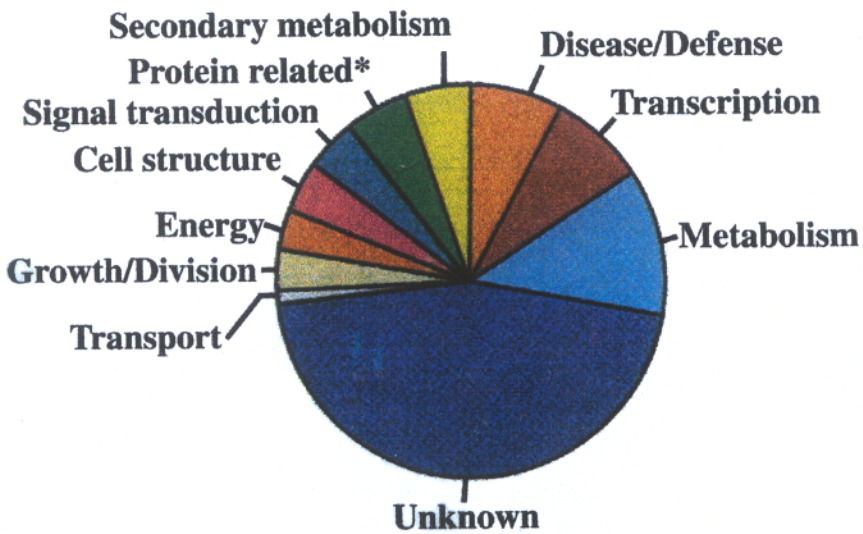
GENOME PROJECTS IDENTIFY ALL GENES IN THE GENOME of AN ORGANISM

Represented Genomes Sequenced to date (2003)

Organism	Genome size (Mb)	Internet address for latest news
Archaea[†]		
Methanococcus jannaschii	1.66	http://www.tigr.org/tdb/mdb/mjdb/mjdb.html
Methanobacterium thermoautotrophicum	1.75	http://www.genomecorp.com/htdocs/sequences/methanobacter/abstract.html
Archaeoglobus fulgidus	2.18	ftp://ftp.tigr.org/pub/data/a_fulgidus
Bacteria[†]		
Mycoplasma genitalium	0.58	http://www.tigr.org/tdb/mdb/mgdb/mgdb.html
Mycoplasma pneumoniae	0.81	http://www.zmbh.uni-heidelberg.de/M-pneumoniae/MP_Home.html
Treponema pallidum	1.14	http://www.tigr.org/tdb/mdb/tpdb/tp_bg.html
Borrelia burgdorferi	1.44	ftp://ftp.tigr.org/pub/data/b_burgdorferi
Aquifex aeolicus	1.55	
Helicobacter pylori	1.66	http://www.tigr.org/tdb/mdb/hpdb/hpdb.html
Haemophilus influenzae	1.83	http://www.tigr.org/tdb/mdb/mdb.html
Synechocystis sp.	3.57	http://kazusa.or.jp/cyano/cyano.html
Bacillus subtilis	4.20	http://www.pasteur.fr/Bio/SubtiList.html
Mycobacterium tuberculosis	4.40	http://www.sanger.ac.uk/Projects/M_tuberculosis/
Escherichia coli	4.64	http://www.genetics.wisc.edu:80/index.html
Eukaryotes		
Saccharomyces cerevisiae	12.1	http://www.mips.biochem.mpg.de/
Arabidopsis thaliana	100	http://genome-www.stanford.edu/Arabidopsis/
Caenorhabditis elegans	100	http://moulon.inra.fr/acedb/acedb.html
Drosophila melanogaster	140	http://flybase.bio.indiana.edu/
Oryza sativa	565	http://www.staff.or.jp/
Homo sapiens	3000	http://gdbwww.gdb.org/
Mus musculus	3300	http://www.informatics.jax.org/

[†] Most of these archaeal and bacterial sequences have already been completed. See also Appendix – Keeping Up to Date.

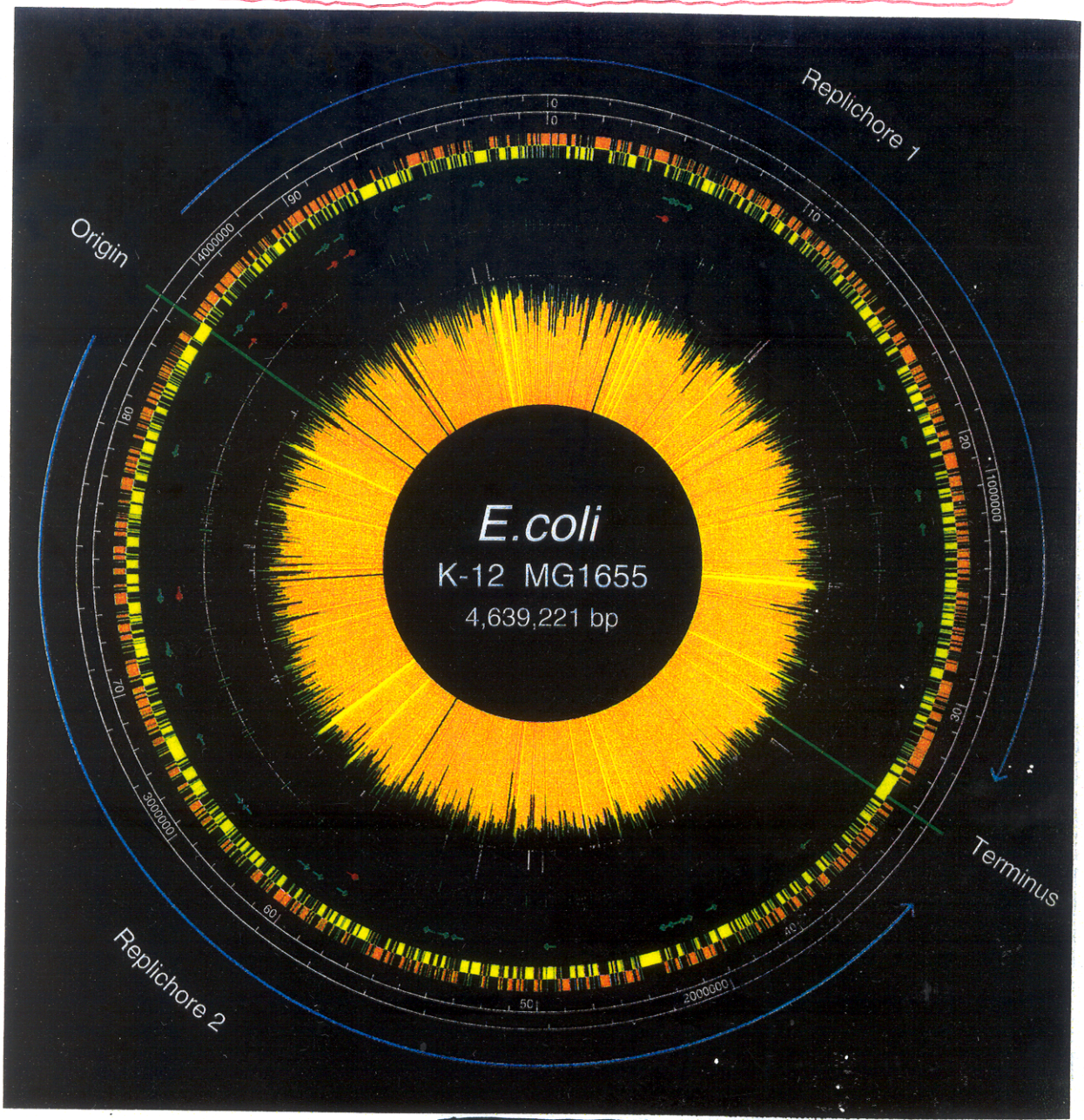
GENOME PROJECTS IDENTIFY genes FOR specific functions



and they tell us "why" organisms are unique!!
e.g. MOUSE & MAN

The E. coli DNA Sequence
4,600,000 bp pairs

Reveals all Genes & Toilet makes this organism Unique!



FACTORY FOR Gene ENGINEERS!

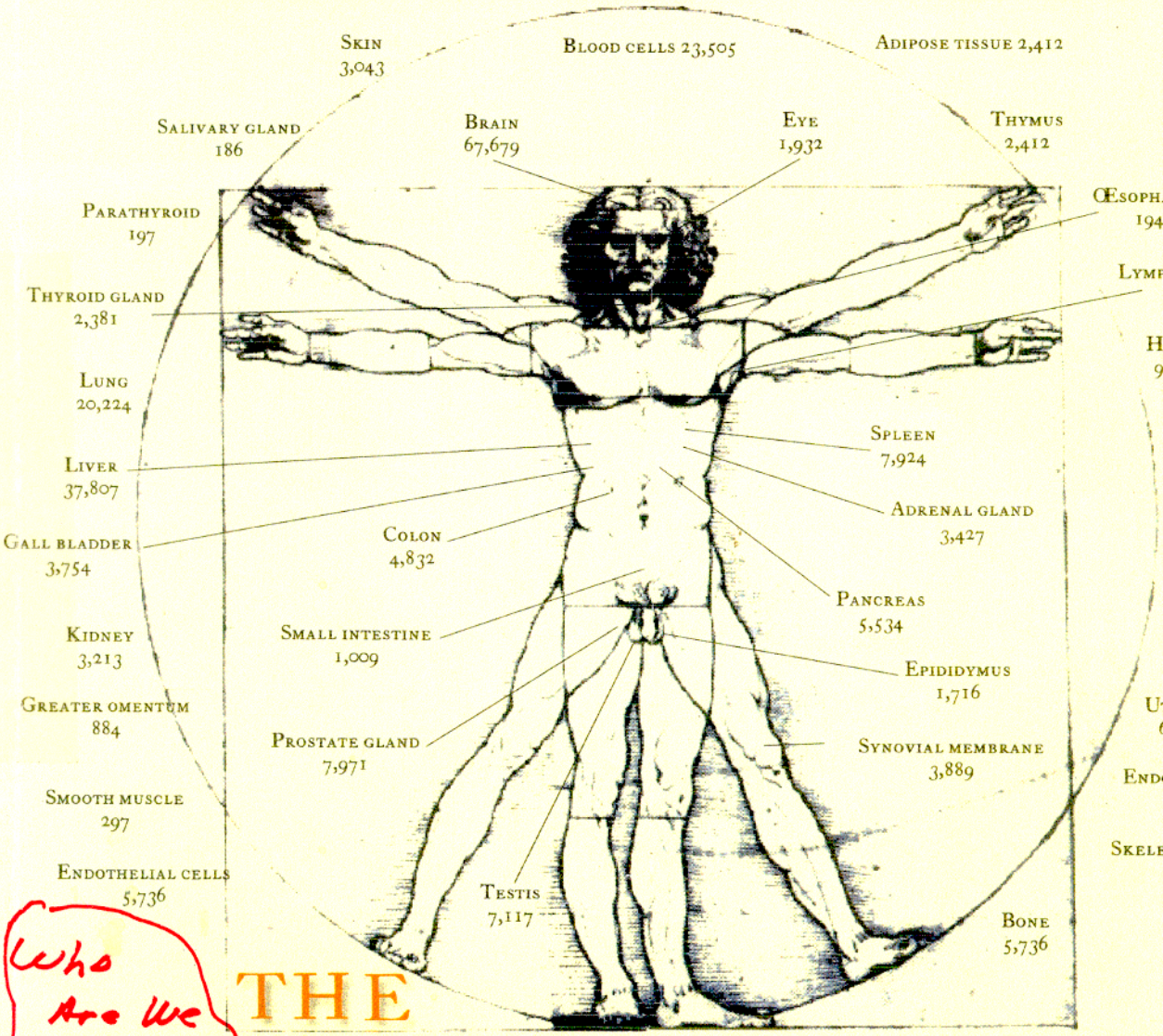
The Sequence Reveals all the genes in the E. coli cell – but NOT the function!

Figure 9.24
Diagram of the DNA sequence organization of *Escherichia coli* strain K-12. The coordinates are given in base pairs as well as in minutes on the genetic map. The coding sequences are shown as gold and yellow bars, which are transcribed in a clockwise (gold) or counterclockwise (yellow) direction. Green and red arrows denote genes for transfer RNAs or for ribosomal RNAs, respectively. The gold rays of the "sunburst" are proportional to the degree of randomness of codon usage in the coding sequences. Genes with the longest rays use the codons in the genetic code almost randomly. The origin and terminus of DNA replication are indicated. Bidirectional replication creates two "replichores." The peaks on the circle immediately outside the sunburst indicate coding sequences with high similarity to previously described bacteriophage proteins. [Courtesy of Frederick R. Blattner and Guy Plunkett III. From F. R. Blattner et al. 1997. *Science* 277: 1453.]

~ 4,000 genes!

The Human Genome Has been Sequenced!

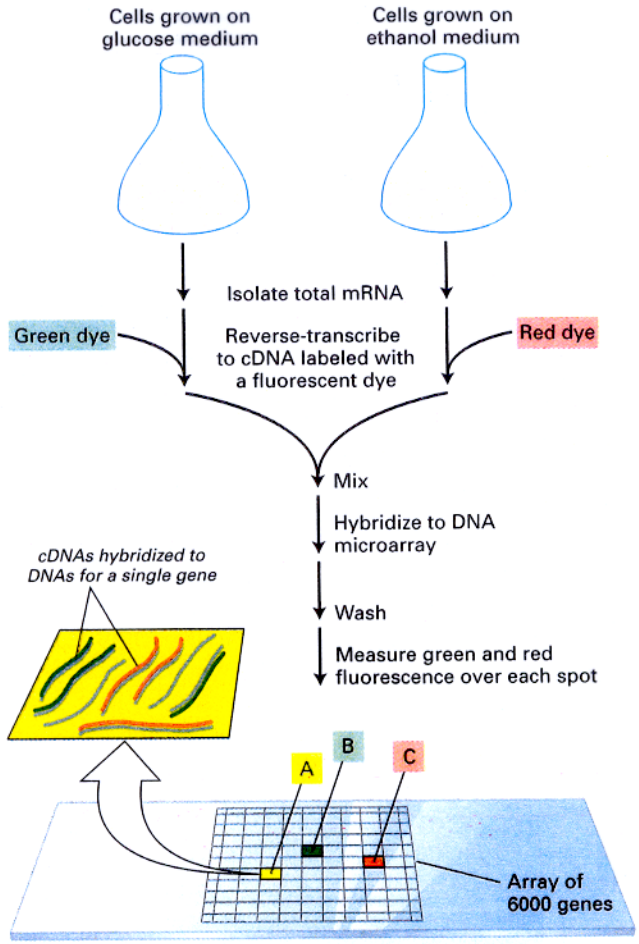
nature
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE



Who Are We + Where Did We Come From?

THE genome DIRECTORY

AND ALL OF YOUR GENES CAN BE STUDIED FOR THEIR ACTIVITY IN CELLS COLLECTIVELY!



A If a spot is yellow, expression of that gene is the same in cells grown either on glucose or ethanol

B If a spot is green, expression of that gene is greater in cells grown in glucose

C If a spot is red, expression of that gene is greater in cells grown in ethanol

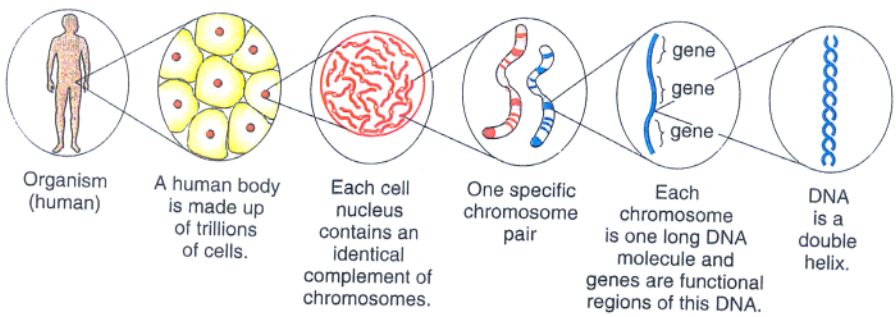
▲ **EXPERIMENTAL FIGURE 9-35 DNA microarray analysis can reveal differences in gene expression in yeast cells under different experimental conditions.** In this example, cDNA prepared from mRNA isolated from wild-type *Saccharomyces* cells grown on glucose or ethanol is labeled with different fluorescent dyes. A microarray composed of DNA spots representing each yeast gene is exposed to an equal mixture of the two cDNA preparations under hybridization conditions. The ratio of the intensities of red and green fluorescence over each spot, detected with a scanning confocal laser microscope, indicates the relative expression of each gene in cells grown on each of the carbon sources. Microarray analysis also is useful for detecting differences in gene expression between wild-type and mutant strains.

DNA Chip → Find which genes are active where e.g., cancer genes

- cancer genes
- heart disease genes
- obesity genes
- hypertension genes
- Aging genes etc., etc.

IT's a NEW ERA of BIOLOGY!

The Human Genome Project will uncover the Functions of all Human Genes



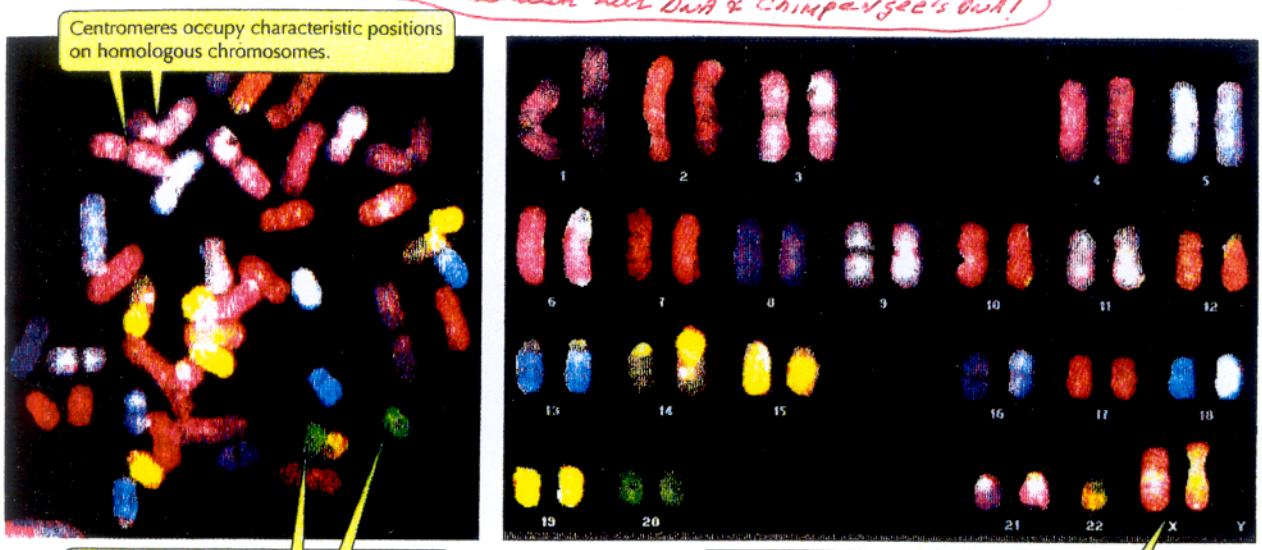
The nucleotide sequence of DNA → Functi

A genome = ALL the genes of an organism

Figure 1-2 Successive enlargements of an organism to focus on the genetic material.

It will tell us why "we" are the same & why we are unique! Already know only ~1%-5% difference between our DNA & chimpanzee's DNA!

Chromosome pair



9.14 Human Cells Have 46 Chromosomes
Chromosomes from a human cell in metaphase of mitosis. In this "chromosome painting" technique, each homologous pair shares a distinctive color. The karyotype on the right is produced by computerized analysis of the image on the left.

What causes color differences?

There are 46 chromosomes in human cells except the egg and sperm which have 23

(1) What accounts for the differences between chromosomes? (2)

(2) Why 2 chromosomes each in Somatic Cell.

Each Chromosome Has a Unique Set of Genes that Direct Unique Biological Processes

Entire Sequence Complete of Every Chromosome!

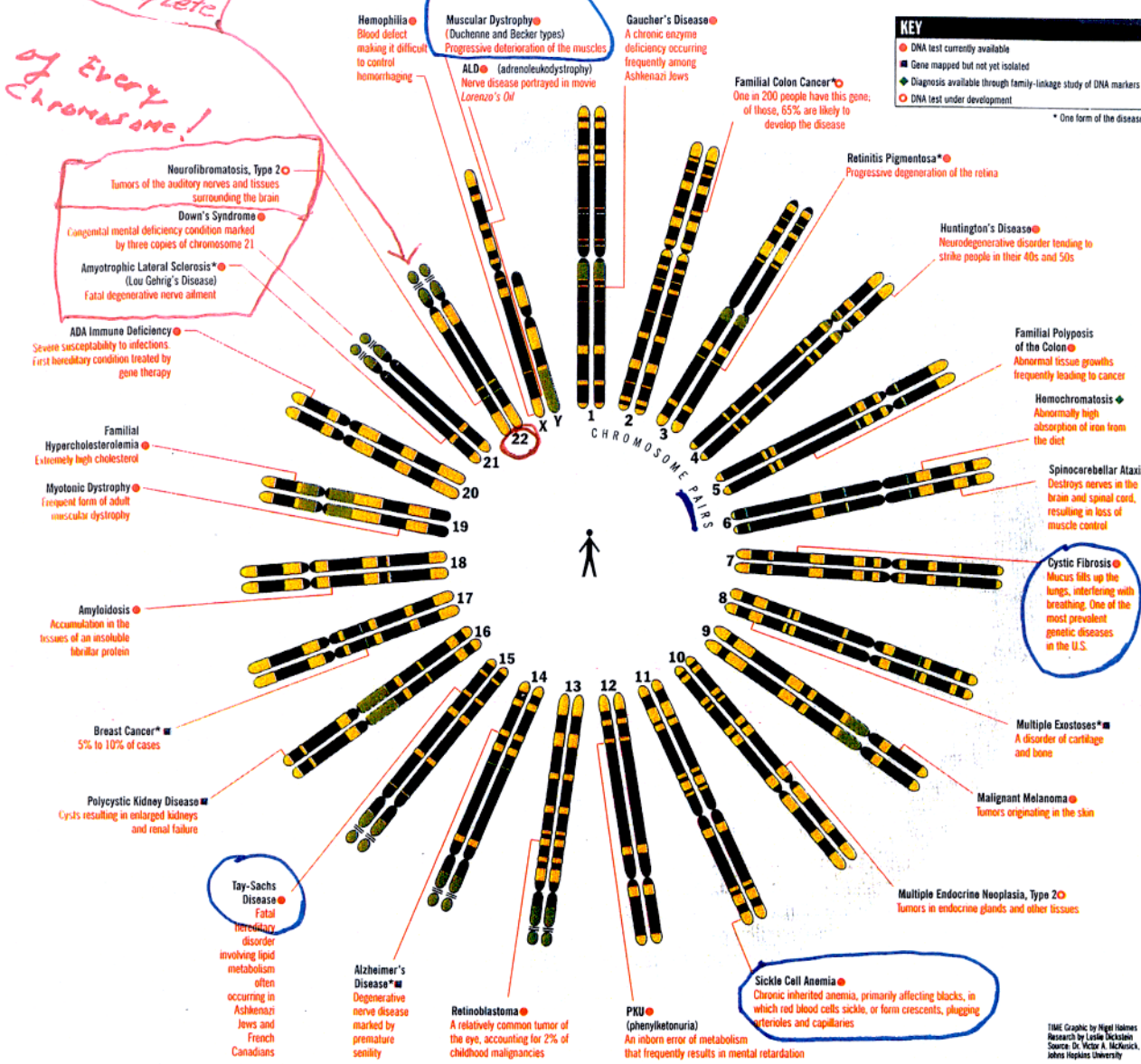


Figure 1-6 The 23 chromosomes of a human being, showing the positions of genes whose abnormal forms cause some of the better-known hereditary diseases. (*Time*)

The Human Genome Project will Reveal the Functions of all human genes & the networks (Logic) that Program all aspects of life from birth to death!

GENOME PROJECTS WILL REVEAL
Ultimately The "Secrets"
of ALL Living
PROCESSES!

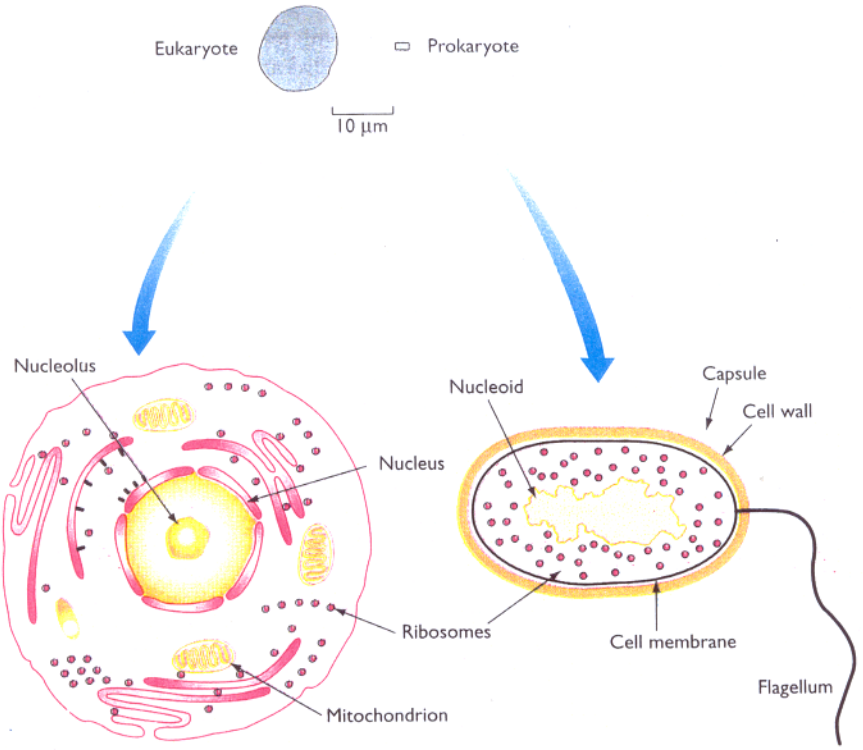


Figure 1.6 Cells of eukaryotes (left) and prokaryotes (right).

The top part of the figure shows a typical human cell and typical bacterium drawn to scale. The human cell is 10 μm in diameter and the bacterium is rod-shaped with dimensions of 1 × 2 μm. The lower drawings show the internal structures of eukaryotic and prokaryotic cells. Eukaryotic cells are characterized by their membrane-bound compartments, which are absent in prokaryotes. The bacterial DNA is contained in the structure called the nucleoid.

COMBINED WITH THE POWER OF GENETIC ENGINEERING
WE WILL HAVE THE ABILITY TO GENERATE
NEW GENES AND ULTIMATELY..... ??

ALLOWS US TO CONTROL AND MANIPULATE
OUR BIOLOGICAL DESTINY!!

The Ultimate Outcome of Genome Projects

1. ALL the genes of major organisms isolated & identified. Use these genes / combine them for any purpose (Medicine, Agriculture).

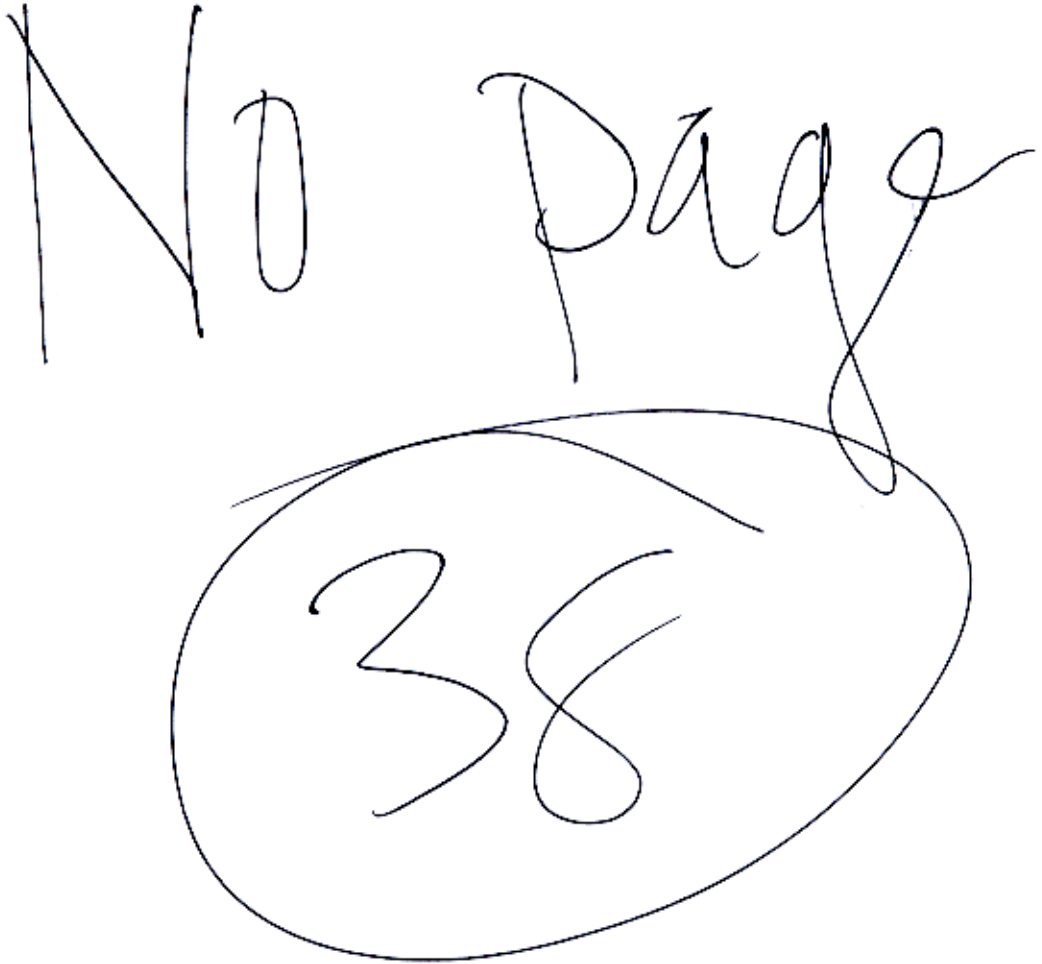
2. ALL of the functions of genes in the cells of Major organisms revealed. What they do to specify traits.

3. The regulatory networks or wiring that controls gene activity from "birth" to "death" revealed. How a child is formed from a fertilized egg cell!

4. The DNA Functions & Networks that direct cells to develop into complex organisms revealed our Biological destiny!!

5. The relationships Between the DNA/Genes of all organisms revealed — What makes a "man a man" and a "mouse a mouse?"

→ Immortality?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THE GENOMICS AND DNA MANIPULATION ERA MEANS IT'S A NEW BALLGAME IN TOWN.....

The ERA of Directing and MANipulating the BIOLOGY of ORGANISMS Has BeGUN

VIRUSES ✓
BACTERIA ✓
FUNGI ✓
PLANTS ✓
ANIMALS (flies, worms, mice, sheep, goats, etc) ✓
HUMANS ✓ ← Even Humans to Correct Lethal Genetic Diseases!

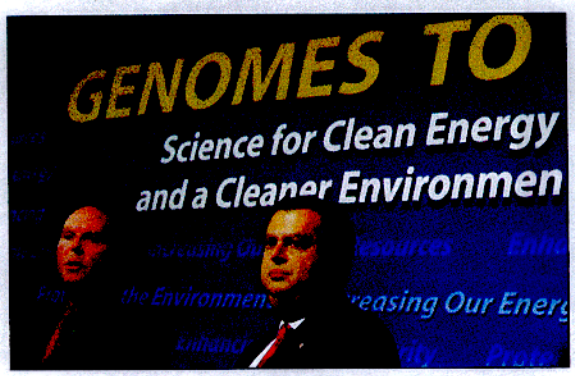
✓ = Have Been Genetically Engineered

And it's Now Possible to synthesize a SMALL CHROMOSOME (VIRUS) from chemical units!

The ultimate in Genetic Engineering: creating "Life" from synthetic Molecules

MOLECULAR BIOLOGY

Venter Cooks Up a Synthetic Genome in Record Time



Stir-and-bake genomes. Venter's (left) success in building a viral genome drew praise from DOE Secretary Abraham.

Generating a synthetic genome by whole genome assembly: ϕX174 bacteriophage from synthetic oligonucleotides

Hamilton O. Smith, Clyde A. Hutchison III†, Cynthia Pfannkoch, and J. Craig Venter‡

Institute for Biological Energy Alternatives, 1901 Research Boulevard, Suite 600, Rockville, MD 20850

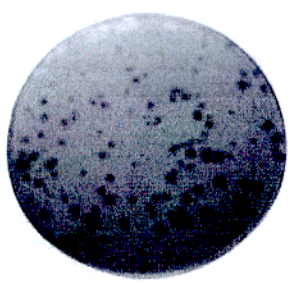


Fig. 4. Plaques of synϕX-A. There appear to be several plaque morphologies: small plaques with sharp borders, medium-sized plaques, and large plaques with fuzzy borders.

What does this Experiment Say about Living Processes?