Genomes & Genomics

Genome, the entire genetic complement of an organism

Genomics, research that addresses all or a substantial portion of an organism's genome

Includes physical mapping & sequencing of all or a large part of a genome or chromosome

Why Study Genomes of Different Organisms?

To understand the genetics behind diseases (*Homo sapien* & *Canis familiarus*)

To learn more about human pathogens & how to prevent or treat their infections (*Clostridium tetani*, *Bacillus anthacis*, & *Haemophilus influenzae*)

Understand & improve the genetics of commercial organisms (*Lactococcus lactis, Oryza sativa, Bos taurus,* & *Gallus gallus*)

To discover the workings of unusual or odd organisms (*Bdellovibrio bacteriovorus & Deinococcus radiodurans*)

To understand phyolegeny

How Many Genomes Have Been Sequenced?

	Completed	Draft	In Progress
Eukaryote	24	129	182
Archaea	46	4	27
Eubacteria	521	414	402
Viral	1703		

(NCBI 9/4/07)

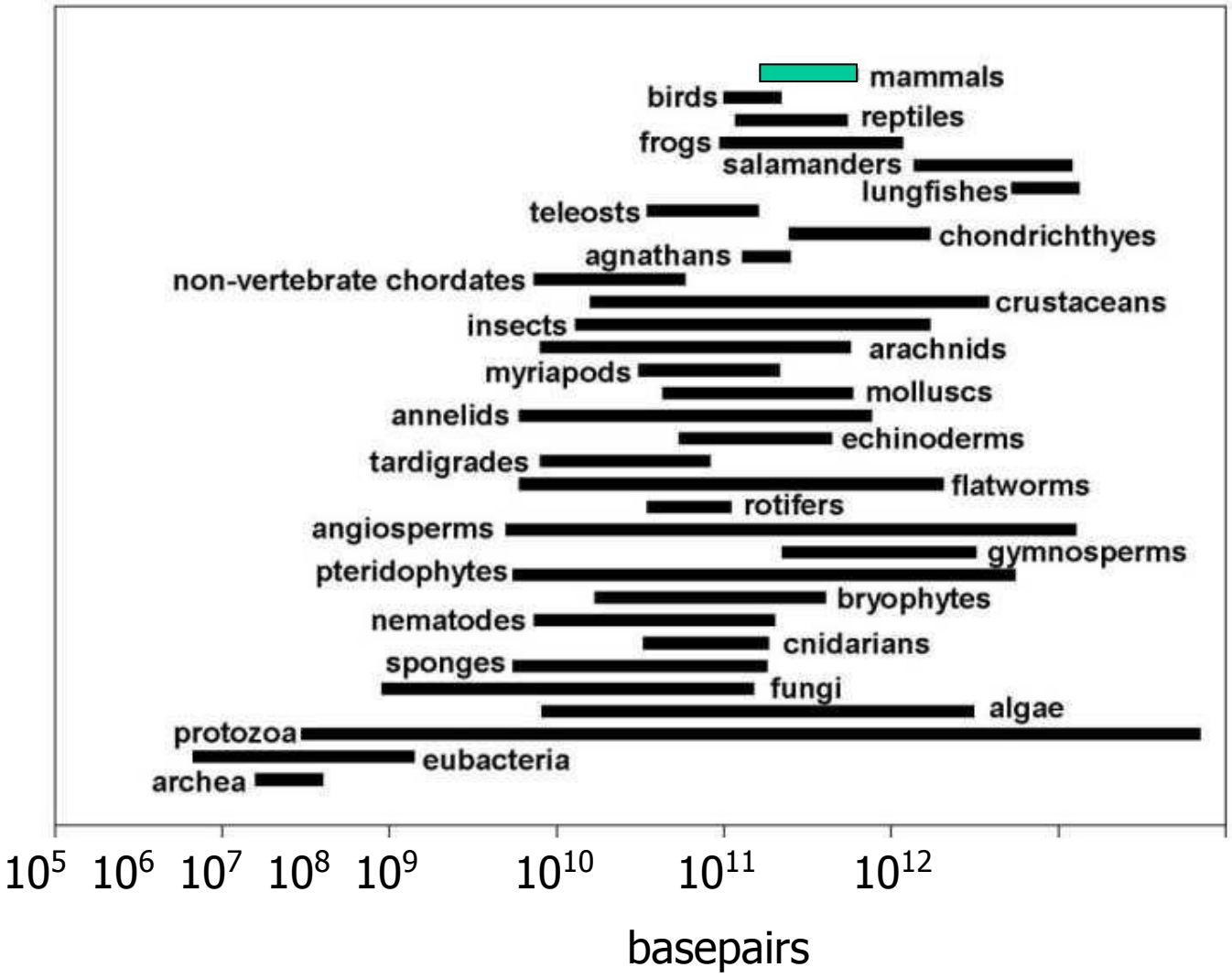
How Do We Measure a Genome?

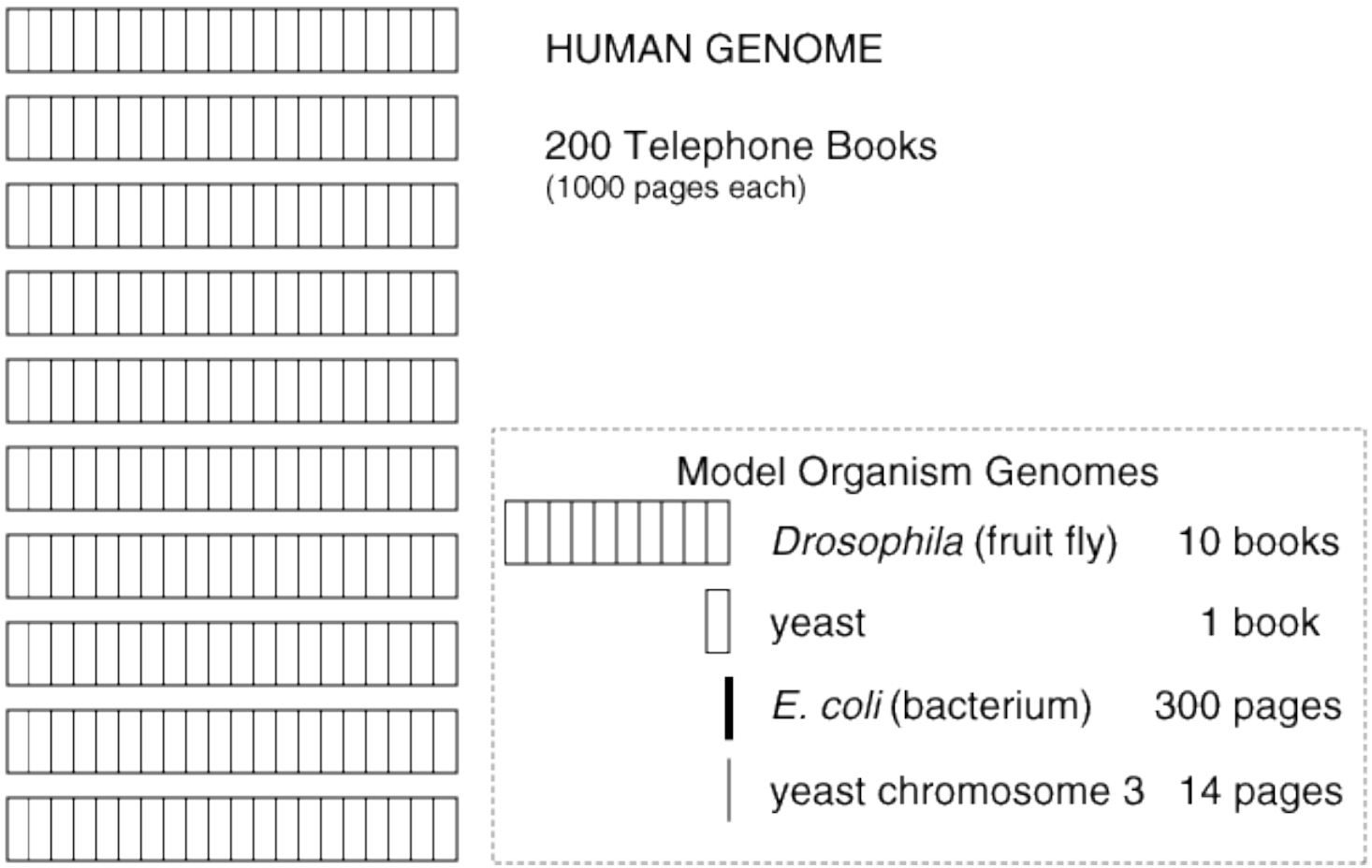
1 base=1 nucleotide=1basepair (bp)
1000bases=1kilobase (Kb)
1000kb=1megabase (Mb)
1000mb=1gigabase (Gb)

Genome Sizes (haploid)

Organism	Genome in Mb
E. coli	4.64
Yeast	12
Nematode	97
Fruit Fly	170
Pufferfish	345
Human	3200
Lungfish	129000

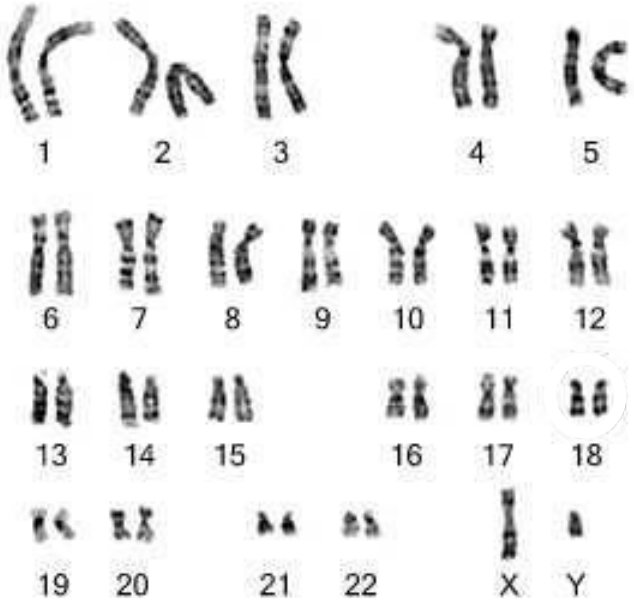
Amount of DNA in a Genome Does Not Correlate with Complexity



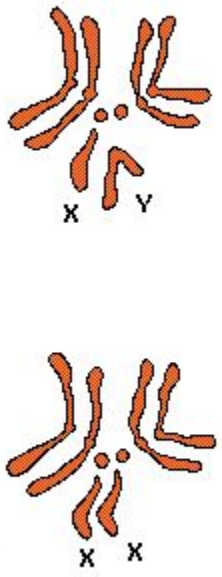


http://www.ornl.gov/sci/techresources/Human_Genome/publicat/primer/fig14.html

Genomes Are Organized Into Chromosomes



Human



Fruit Fly

http://www.chromosome18.org/graphics/Slide.gif
http://uk.encarta.msn.com/media_121636626/Fruit_Fly_Chromosomes.html

Chromosome Number Is Species Specific

	Diploid Number 2n
Human	46
Mouse	40
Fruit Fly	8
Dog	78
Arabidopsis	10
Corn	20
Yeast	32
Crayfish	200

How many genes do we have?

Original estimate was between 50 000 to 100 000 genes

We now think human have ~ 25 000 genes

How does this compare to other organisms?

Mice have ~30 000 genes

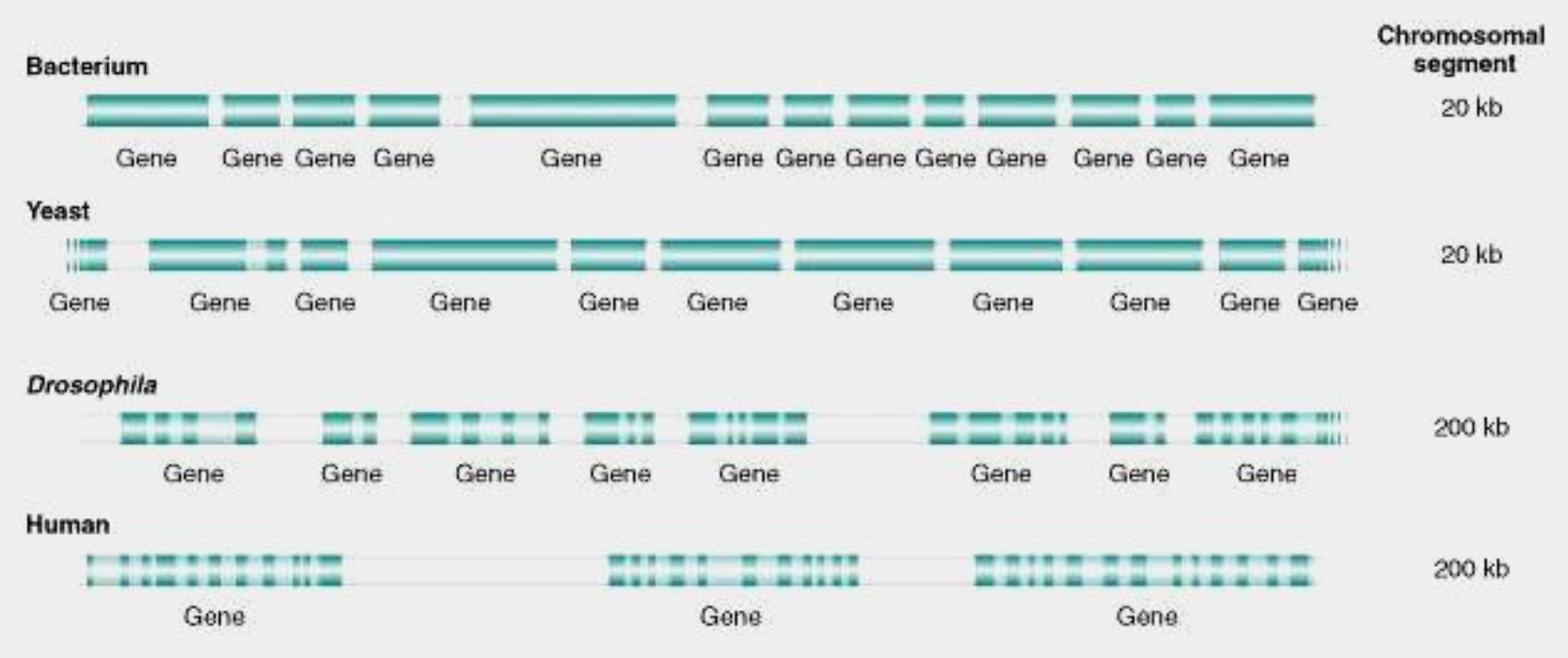
Pufferfish have ~35 000 gene

The nematode (*C. elegans*), has ~19 000

Yeast (*S. cerevisiae*) there are ~6000 genes

The microbe responsible for tuberculosis has ~4000

Gene Spacing in Various Species



http://www.mun.ca/biology/desmid/brian/BIOL2250/Week_Two/genespac.jpg

Even the Amount of DNA a Gene Spans Differs Amongst Species

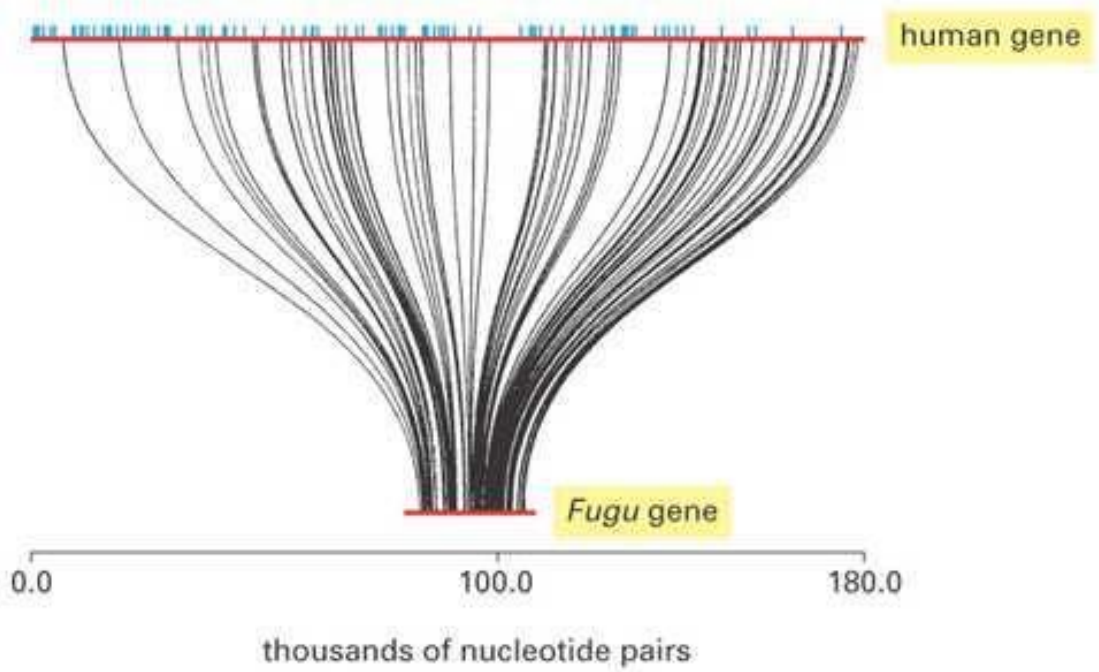


Figure 9-21 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Comparative Genomics

Table 1: Comparison of Selected Genomes

Organism	Approximate Size of Genome (Date Completed)	Number of Genes	Approximate Percentage of Genes Shared with Humans	Web Access to Genome Databases
Bacterium (*Escherichia coli*)	4.6 million bp (1997)	4,403	Not determined	http://www.genome.wisc.edu/
Fruit fly (*Drosophila melanogaster*)	165 million bp (2000)	~13,600	50%	www.fruitfly.org/sequence.html http://Flybase.bio.indiana.edu/
Humans (*Homo sapiens*)	3 billion bp (February 2001)	30,000–40,000	100%	http://www.ornl.gov/hgmis/
Mouse (*Mus musculus*)	~3 billion bp (to be completed in 2001)	~35,000	~90%	http://www.informatics.jax.org/ http://www-genome.wi.mit.edu /genome_data/mouse /mouse_index.html
Plant (*Arabidopsis thaliana*)	125 million bp (2000)	~25,000	Not determined	http://www.arabidopsis.org/
Roundworm (*Caenorhabditis elegans*)	97 million bp (1998)	19,099	40%	www.genome.wustl.edu/gsc.C_elegans
Yeast (*Saccharomyces cerevisiae*)	12 million bp (1996)	~6,000	31%	http://genome-www.stanford.edu /Saccharomyces/

Source: Howard Hughes Medical Institute (2001), *The Genes We Share with Yeast, Flies, Worms, and Mice: New Clues to Human Health and Disease.*

Yeast

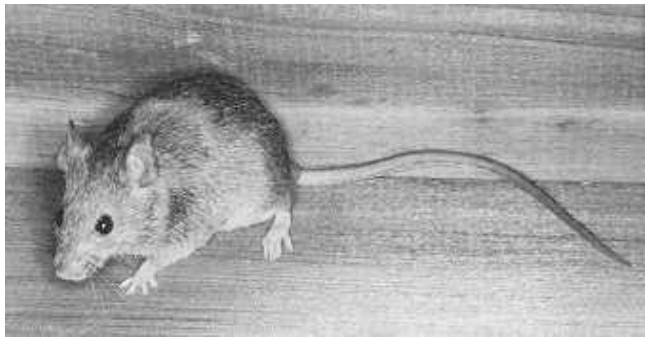
- 70 human genes are known to repair mutations in yeast
- Nearly all we know about cell cycle and cancer comes from studies of yeast
- Advantages:
  - fewer genes (6000)
  - few introns
  - 31% of yeast genes give same products as human homologues

Drosophila

- nearly all we know of how mutations affect gene function come from *Drosophila* studies
- We share 50% of their genes
  - 61% of genes mutated in 289 human diseases are found in fruit flies
  - 68% of genes associated with cancers are found in fruit flies
- Knockout mutants
- Homeobox genes

C. elegans

- 959 cells in the nervous system
- 131 of those programmed for apoptosis
- apoptosis involved in several human genetic neurological disorders
    - Alzheimers
    - Huntingtons
    - Parkinsons

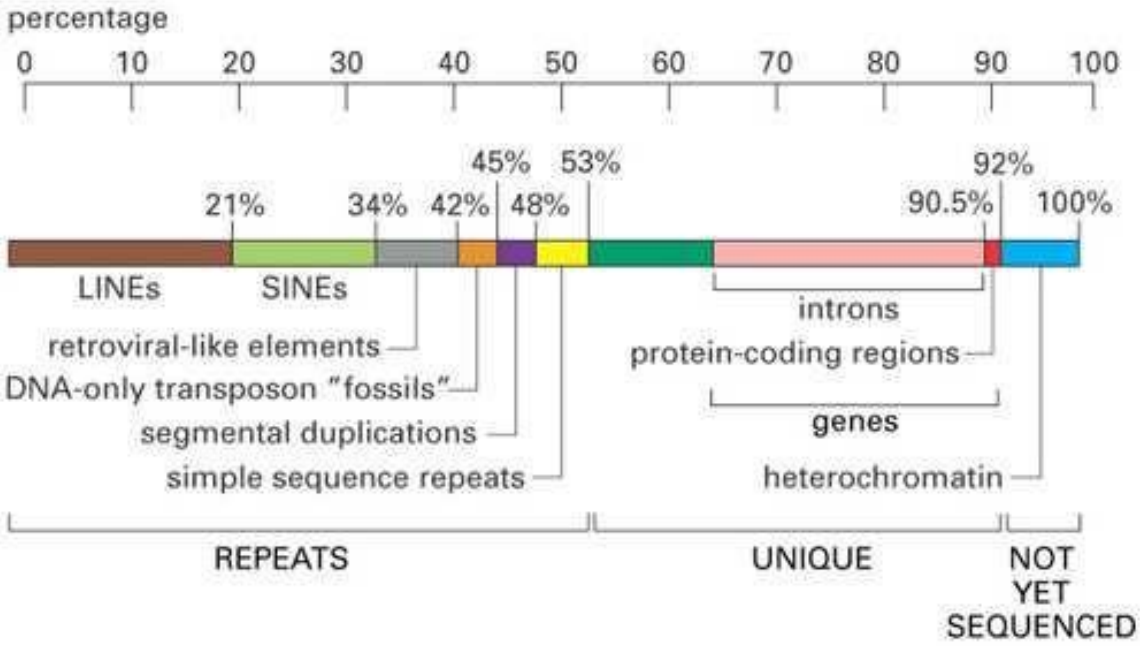


Mouse

- known as “mini” humans
  - Very similar physiological systems
  - Share 90% of their genes

What is the rest of the human genome made up of?

- Regulatory regions of DNA that turn genes on or off
- Repetitive DNA sequences:
  - Tandem Repetitive Sequences (~10%)
    - Microsatellite DNA: 2 to 4bp long repeats
    - Minisatellite DNA: 20bp or longer repeats
    - Macrosatellite DNA: megabase long repeats
  - Transposable elements *SINEs* and *LINEs* 35%
    - Retroviral fossils



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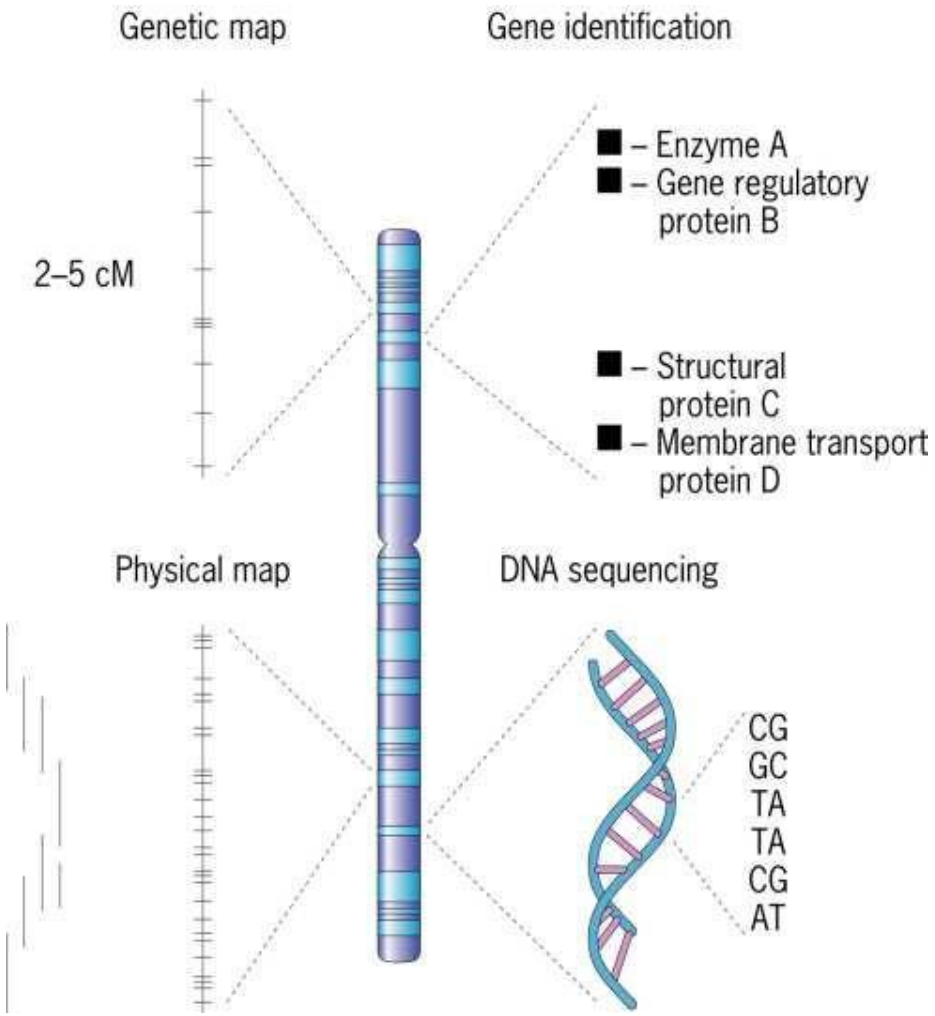
Genetic vs. Physical Mapping



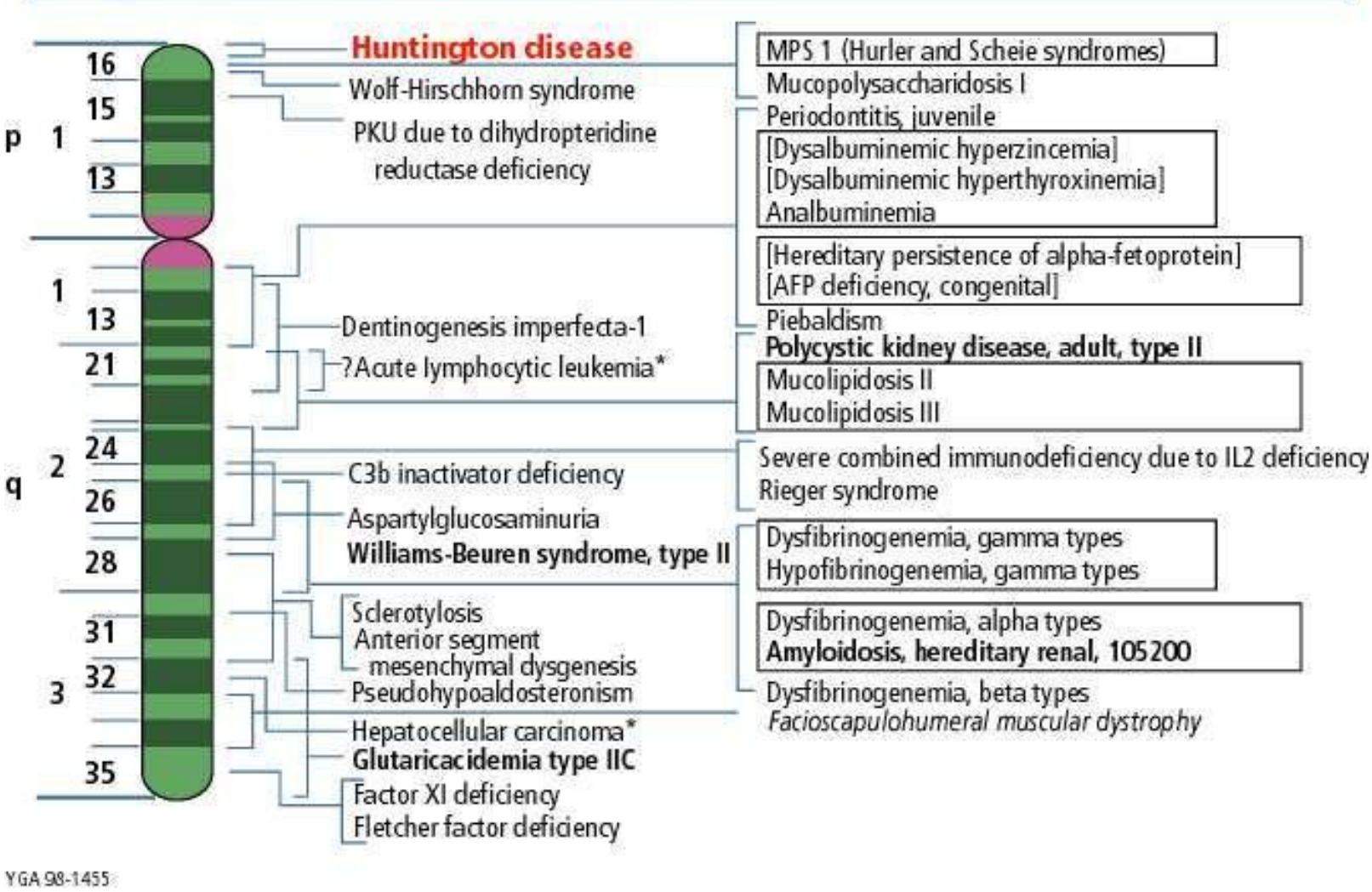
Genetic mapping based on genetic techniques, maps show the positions of diseases or traits based on recombination frequencies

Genetic techniques include cross-breeding experiments or, the examination of family histories (pedigrees)

Physical mapping uses molecular biology techniques to examine DNA molecules directly to construct maps showing the positions of sequence features, including genes

Physical techniques include DNA restriction enzyme analysis & fluorescent tagging of chromosomal regions

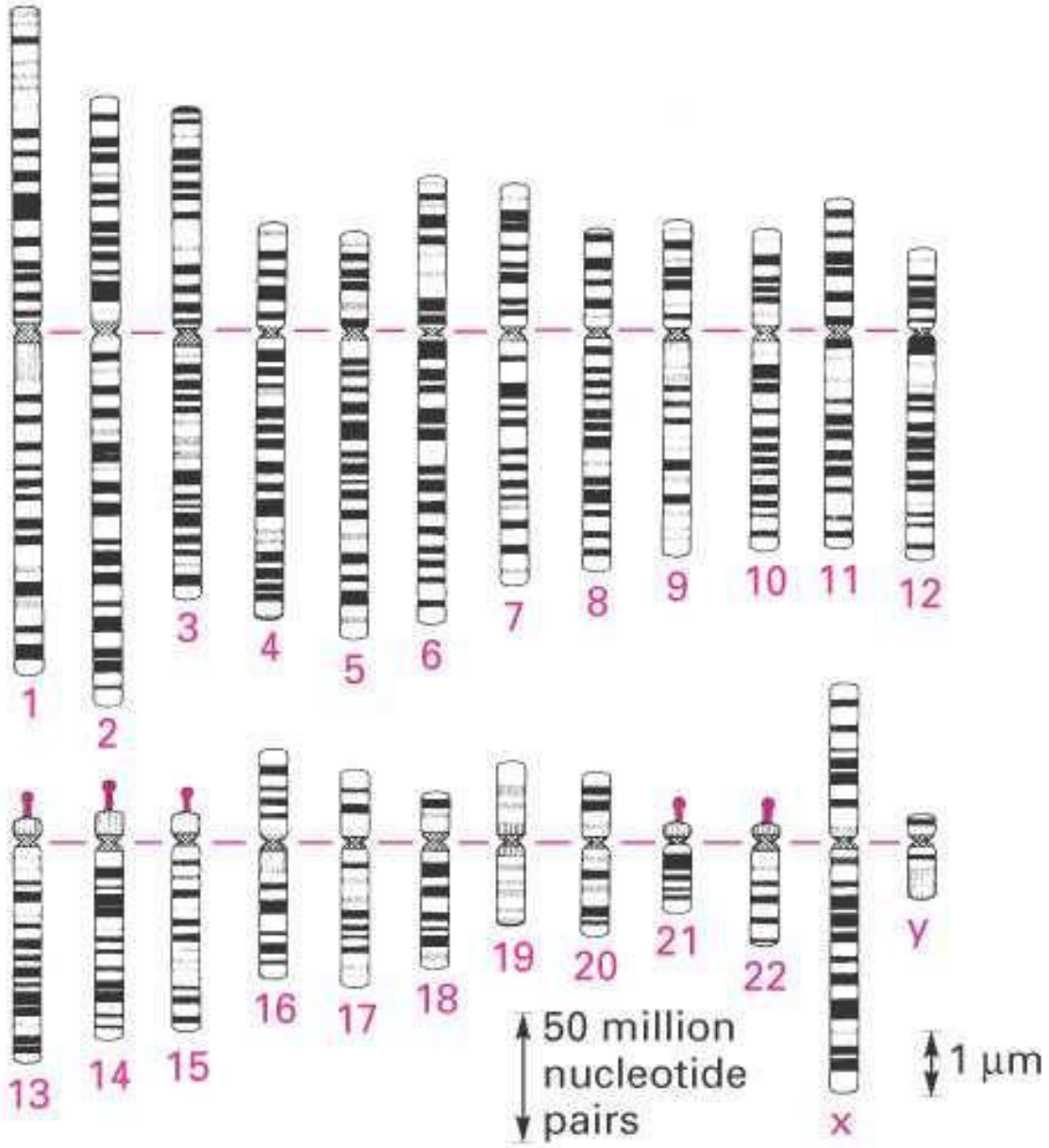
Genetic Map showing the location of disease genes on human chromosome 4



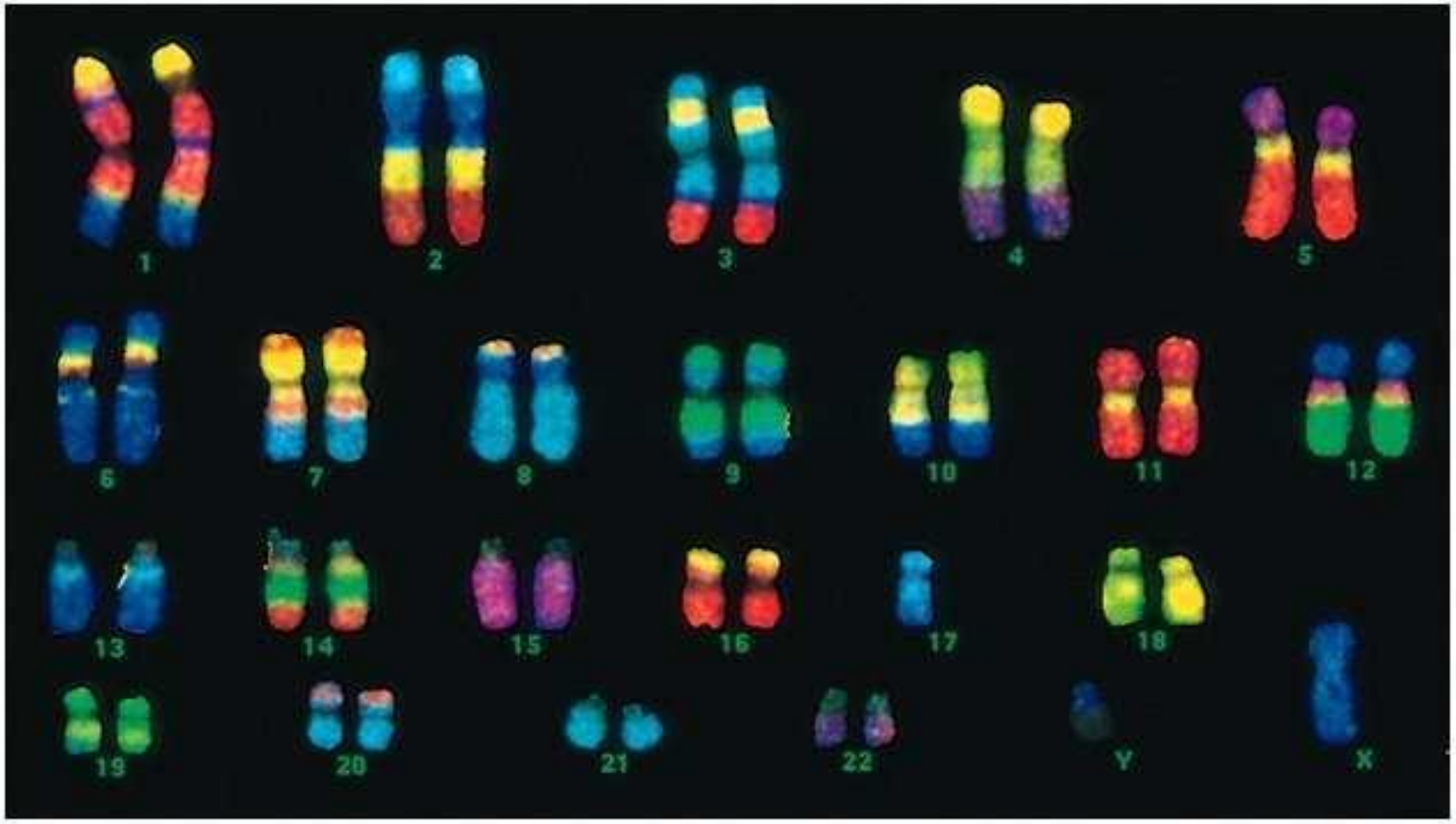
http://www.ornl.gov/TechResources/Human_Genome/graphics/slides/images/98-1455.jpg

Human chromosomes stained to show bands of different DNA

These bands are the roughest markers for physical mapping

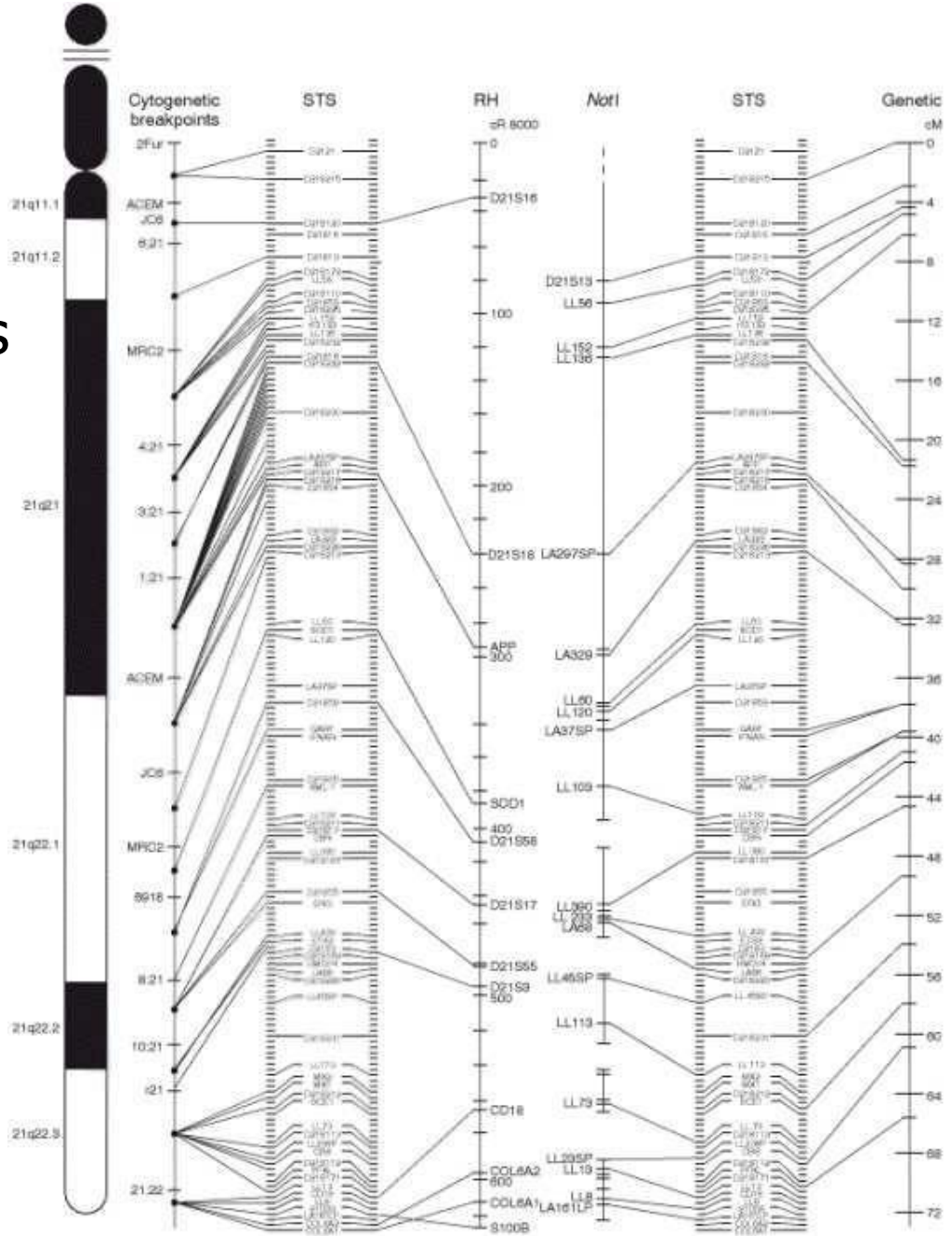


Fluorescent Labeling of Chromosomes



http://www.mun.ca/biology/scarr/FISH_chromosome_painting.htm

Types of Physical Maps For Chromosome 21



http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=hmg.figgrp.1556

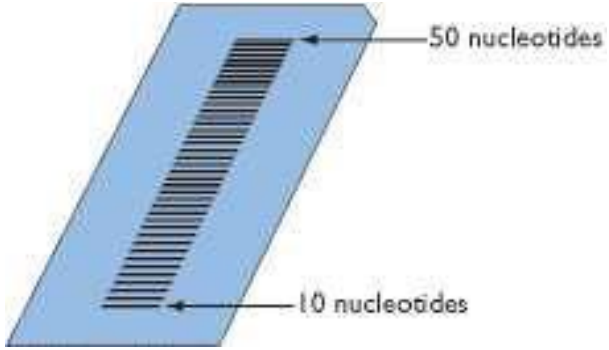
The more markers better the resolution, the more useful the map

http://freepages.genealogy.rootsweb.com/~patafordgenealogy/images/usmaphyperlinks.jpg
http://socialstudies.ccswebacademy.net/CivicsEconomicsJenkins/images/Map_Outline_US_Outline.jpg

DNA Sequencing

Polyacrylamide gel electrophoresis can resolve ssDNA molecules that differ in length by just one nucleotide

A banding pattern is produced after separation of ssDNA molecules by denaturing polyacrylamide gel electrophoresis



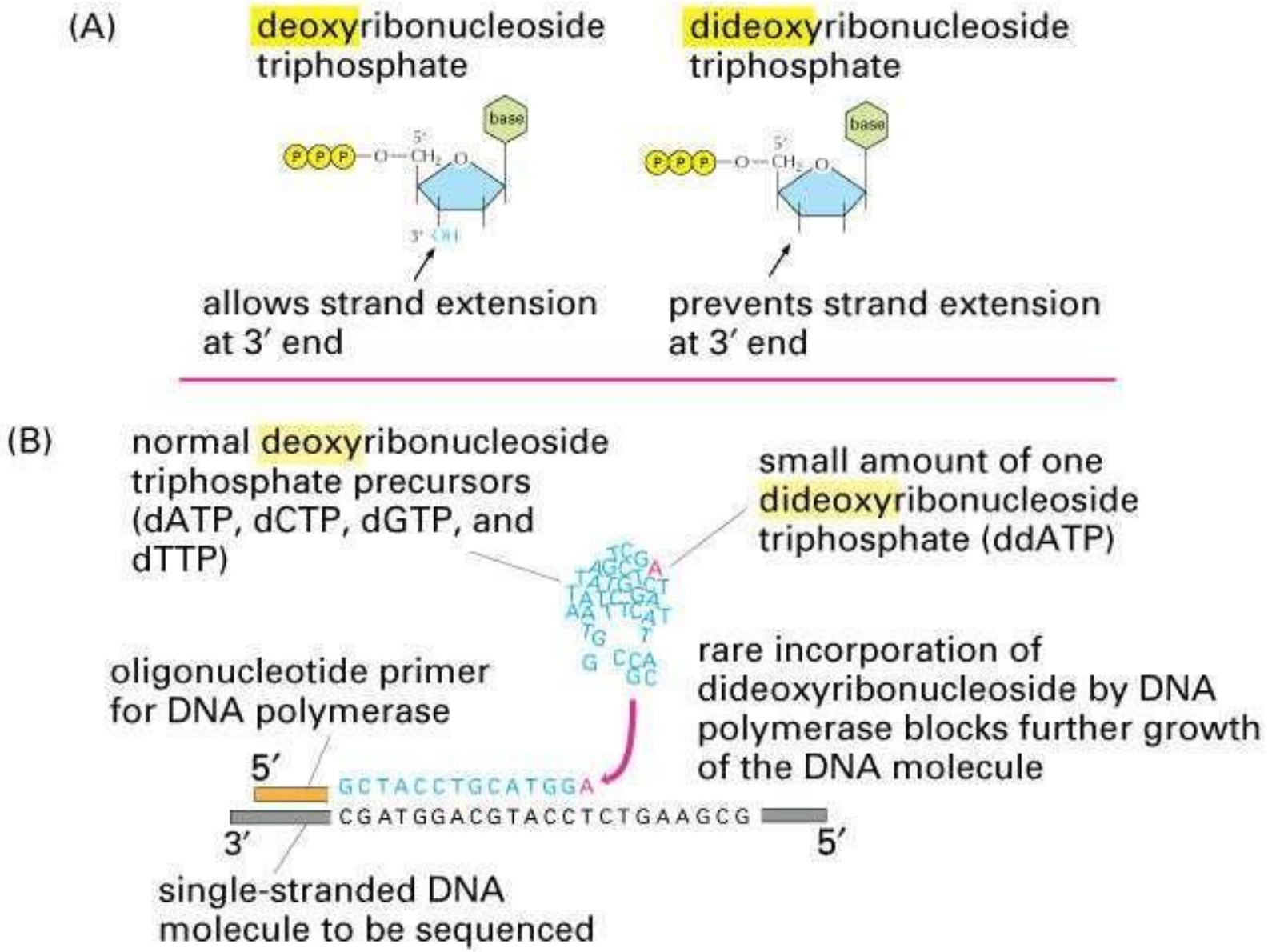
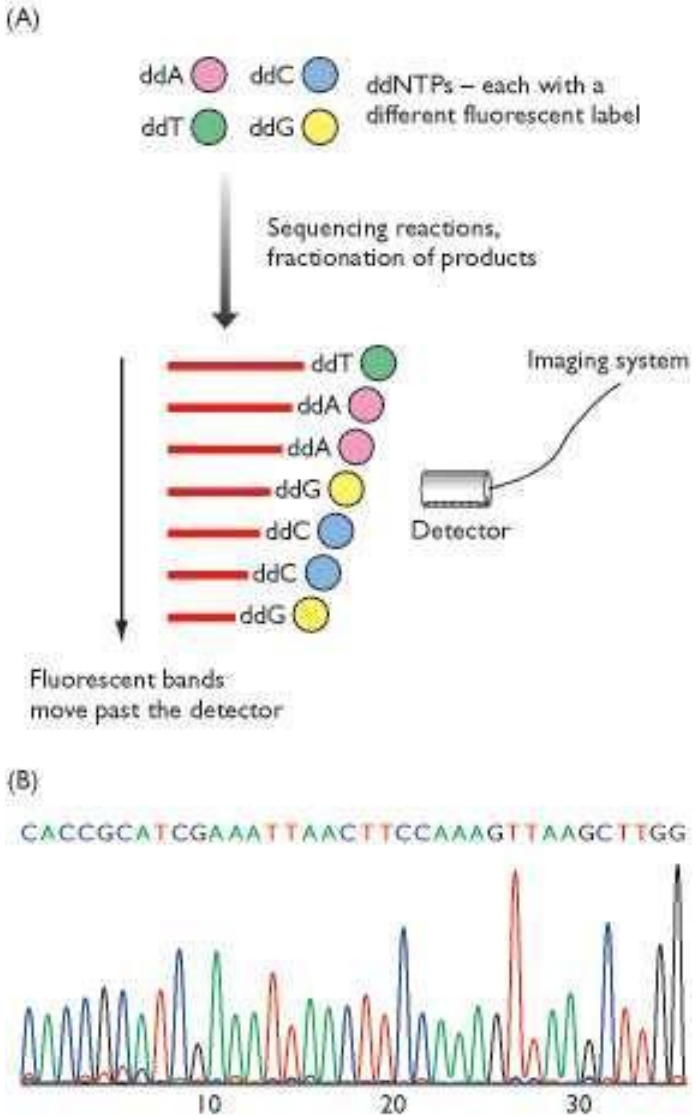
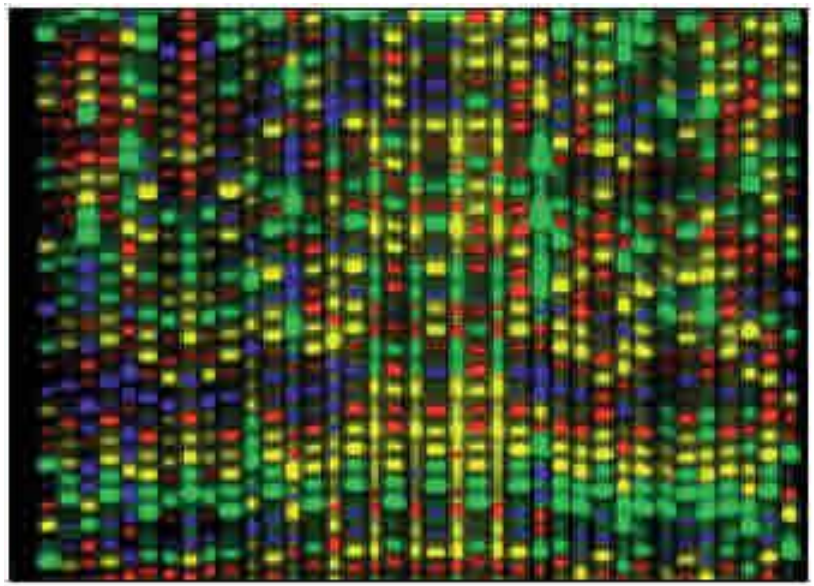


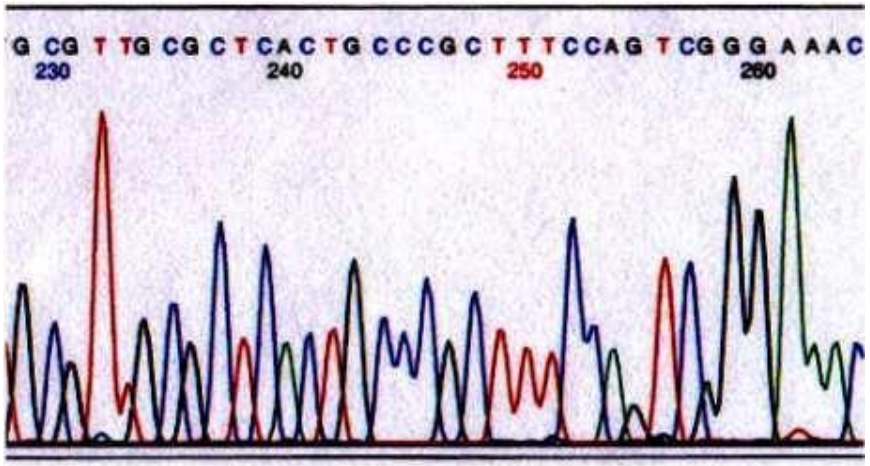
Figure 10-7 part 1 of 2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Automatic Sequencing Machines use fluorescent dyes



Fluorescent Dye Dideoxy-sequencing





DNA Sequencers in Action

http://www.ornl.gov/TechResources/Human_Genome/graphics/slides/ttseqfacility.html

First Complete Sequence of a Free-Living Organism

1995, the *Haemophilus influenzae* genome sequenced

Genome size=1830 kb

1st genome sequenced using the shotgun method

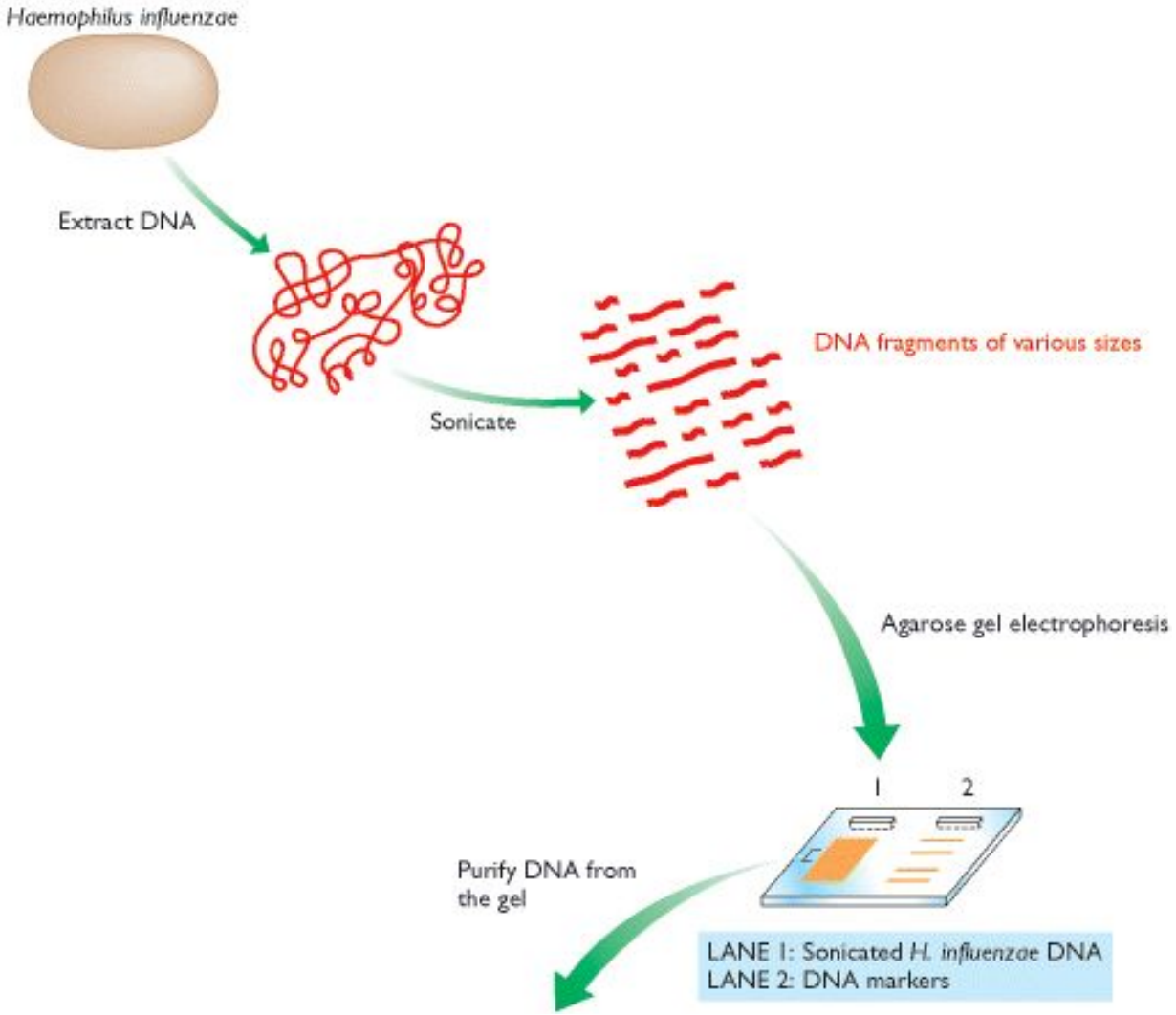
28,643 sequencing experiments totaling 11,631,485 bp

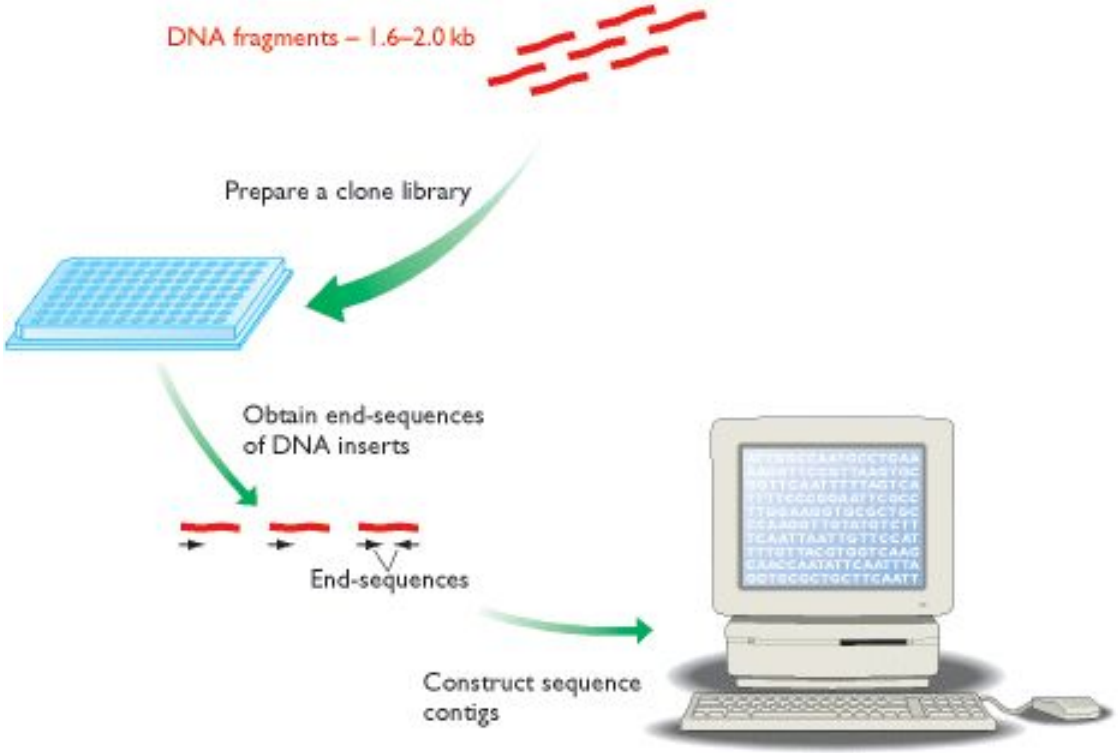
This equaled 6x the length of the *H.* influenzae genome

Sequence assembly 30 hrs on a computer with 512 Mb of RAM

Resulted in 140 lengthy contiguous sequences

Each sequence contig represented, non-overlapping portion of the genome


Haemophilus influenzae
Extract DNA
Sonicate
DNA fragments of various sizes
Agarose gel electrophoresis
1
2
Purify DNA from the gel
LANE 1: Sonicated H. influenzae DNA
LANE 2: DNA markers


DNA fragments – 1.6–2.0 kb
Prepare a clone library
Obtain end-sequences
of DNA inserts
End-sequences
Construct sequence
contigs

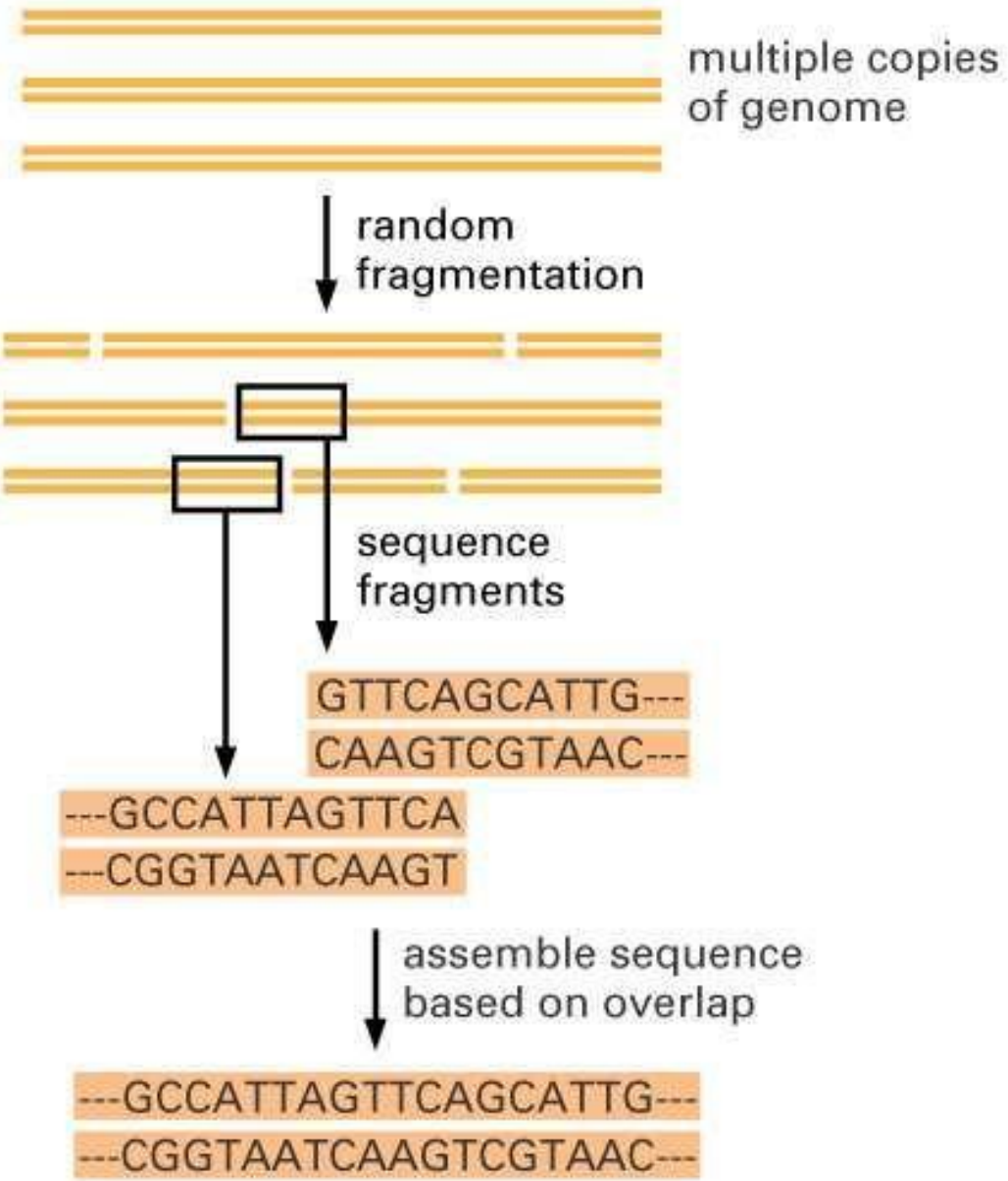


Figure 10-9 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Human Genome Project

1st proposed by the DoE 1984

By 1990, the Human Genome Project was launched

The Human Genome Organization (HUGO) was founded to provide a forum for international coordination of genomic research

The program was proposed to include:

The creation of genetic & physical maps to be used in the generation of a complete genome sequence

First Steps of the Human Genome Project

1) Construct genetic & physical maps of the haploid human & mouse genomes

These would provide key tools for identification of disease genes and anchoring points for genomic sequence

2) Sequence the yeast and worm genomes, as well as targeted regions of mammalian genomes

Sequencing Plan of HUGO

1) Isolate each human chromosome

2) Physical mapping of each chromosome

   The banding pattern of visible through staining

   Location of known genes already mapped

   Location of restriction enzyme sites

Chromosome fragmented into large pieces of DNA and inserted into BAC or YAC libraries

Fragments overlap such that they can be ordered into a rough assembly of the chromosome

DNA from 5 humans
2 males, 3 females
2 caucasians, one each of asian, african, hispanic

Each YAC or BAC is fragmented into smaller 1 to 2 kb pieces of DNA which are sequenced

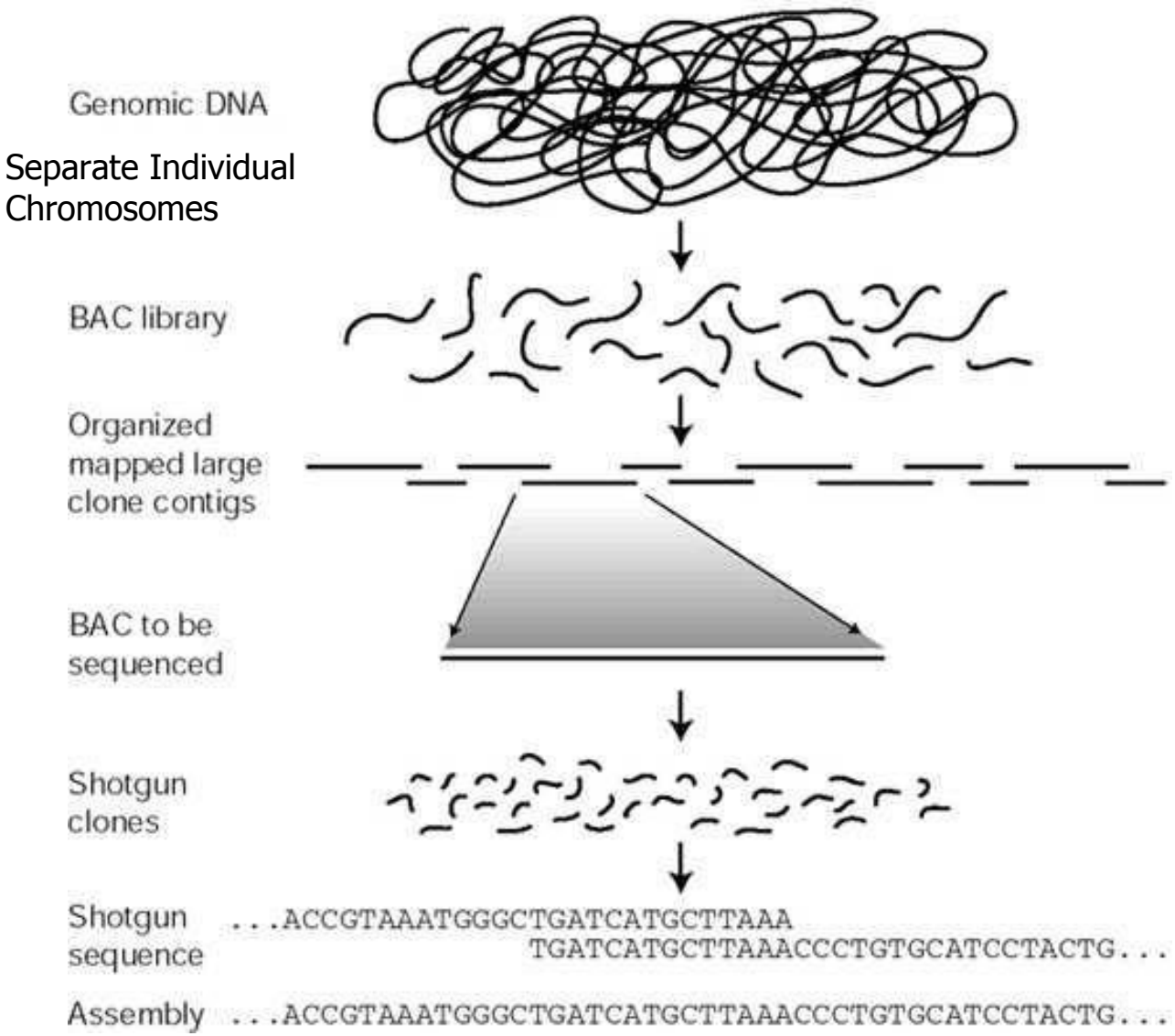
Each of these fragments slightly overlaps with each other

A computer takes the DNA sequences & looks for regions of overlap these are connected to form a sequence contig for the entire BAC or YAC

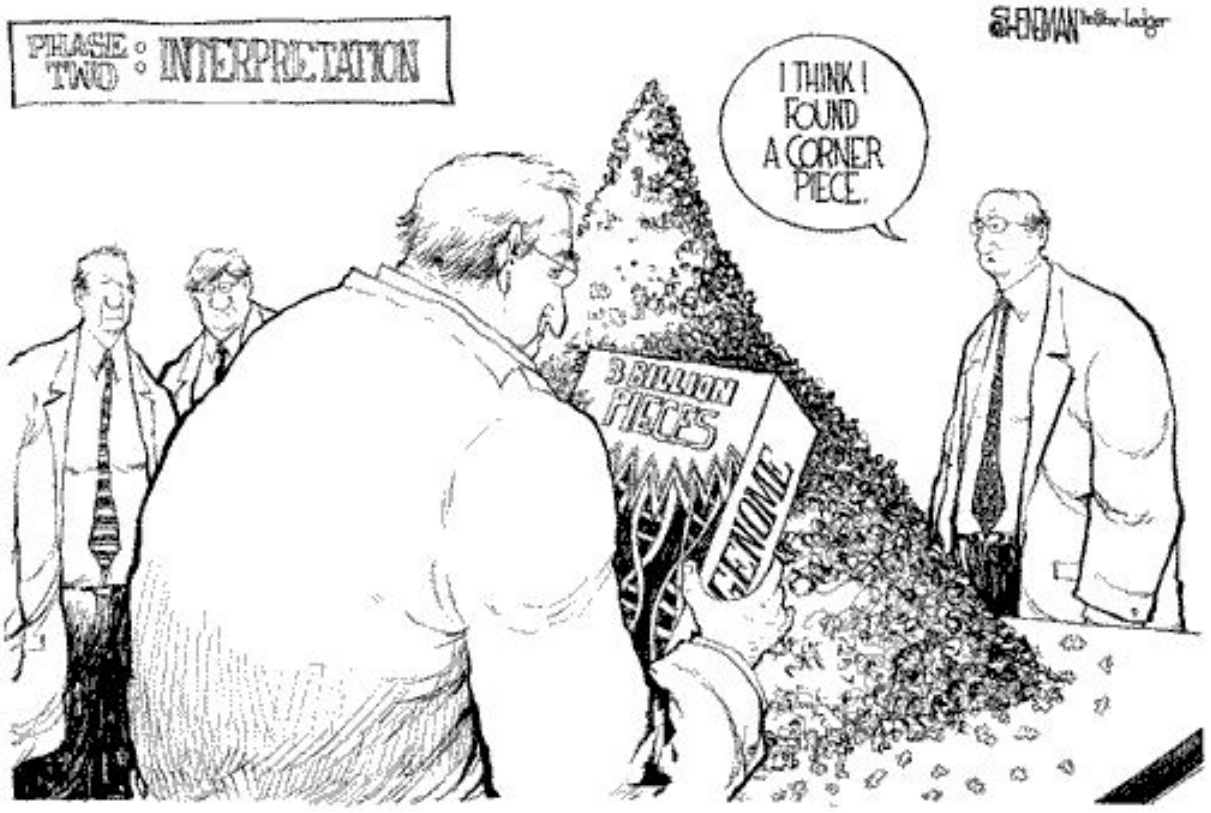
The sequence of all the YACs or BACs are assembled through the same process to give the sequence of the chromosome

This is repeated for all 22 chromosomes plus the X & Y

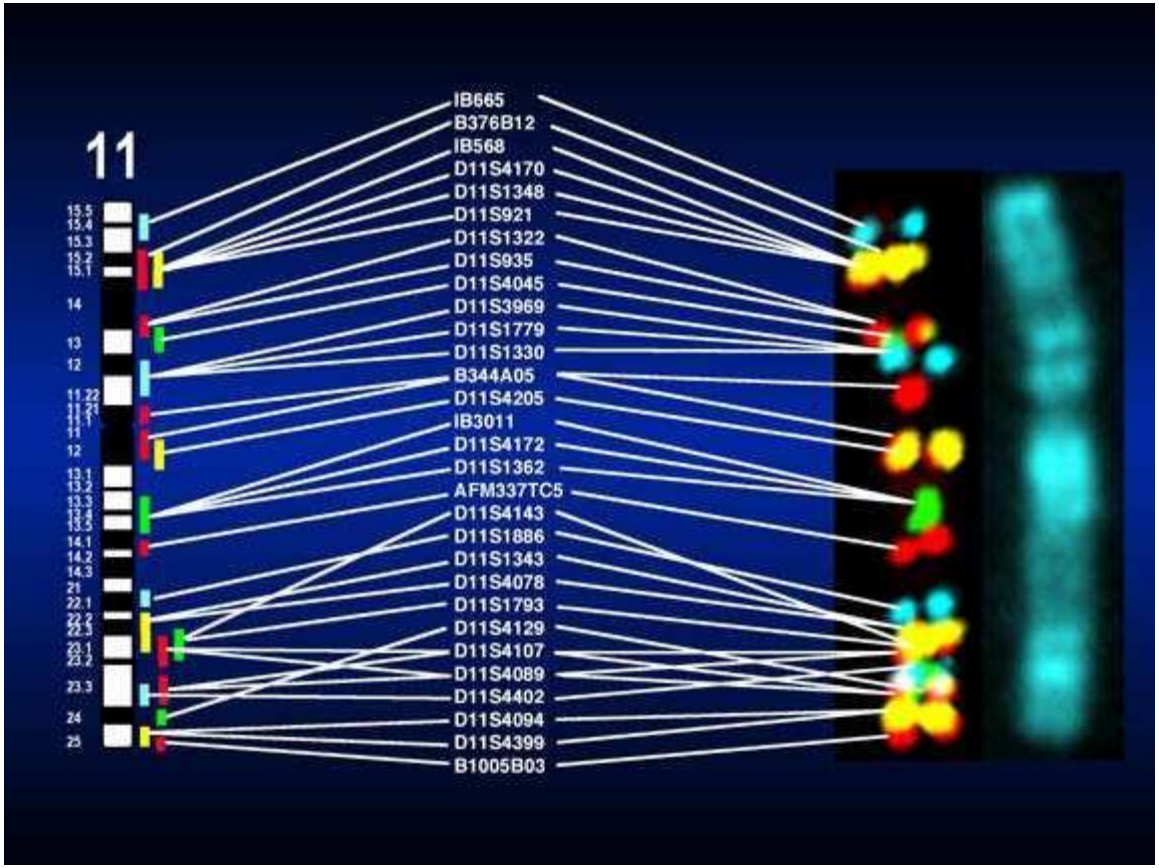
Hierarchical Shotgun Approach



http://www.genome.ou.edu/3653/3653-101705.html


PHASE TWO: INTERPRETATION
SHERMAN
I THINK I FOUND A CORNER PIECE.
3 BILLION PIECES
GENOME

Chromosome 11 BACs



http://www.csmc.edu/csri/korenberg/chroma11.html

Human Genome Whole-Genome Shotgun Method

1999, Celera Genomics, set out to sequence the human genome using a whole-genome shotgun method - more riskier - goal to patent some seq.

There would be no isolation of individual chromosomes & no subcloning into BACs or YACs

They skipped straight to the 1 to 2 kb fragments

The $300 million Celera effort was intended to proceed at a faster pace and at a fraction of the cost of the roughly $3 billion HUGO project.

Dr. Craig Venter (founder) Celera Genomics

14.8-billion bp of DNA sequence was generated over 9 months

This equaled 5x the human genome

Resulting sequence contigs spanned >99% of the genome

In March 2000, President Clinton announced that the genome sequences could not be patented, and should be made freely available to all researchers. The statement sent Celera's stock plummeting.

The competition proved to be very good for the project, spurring the public groups to modify their strategy in order to accelerate progress.

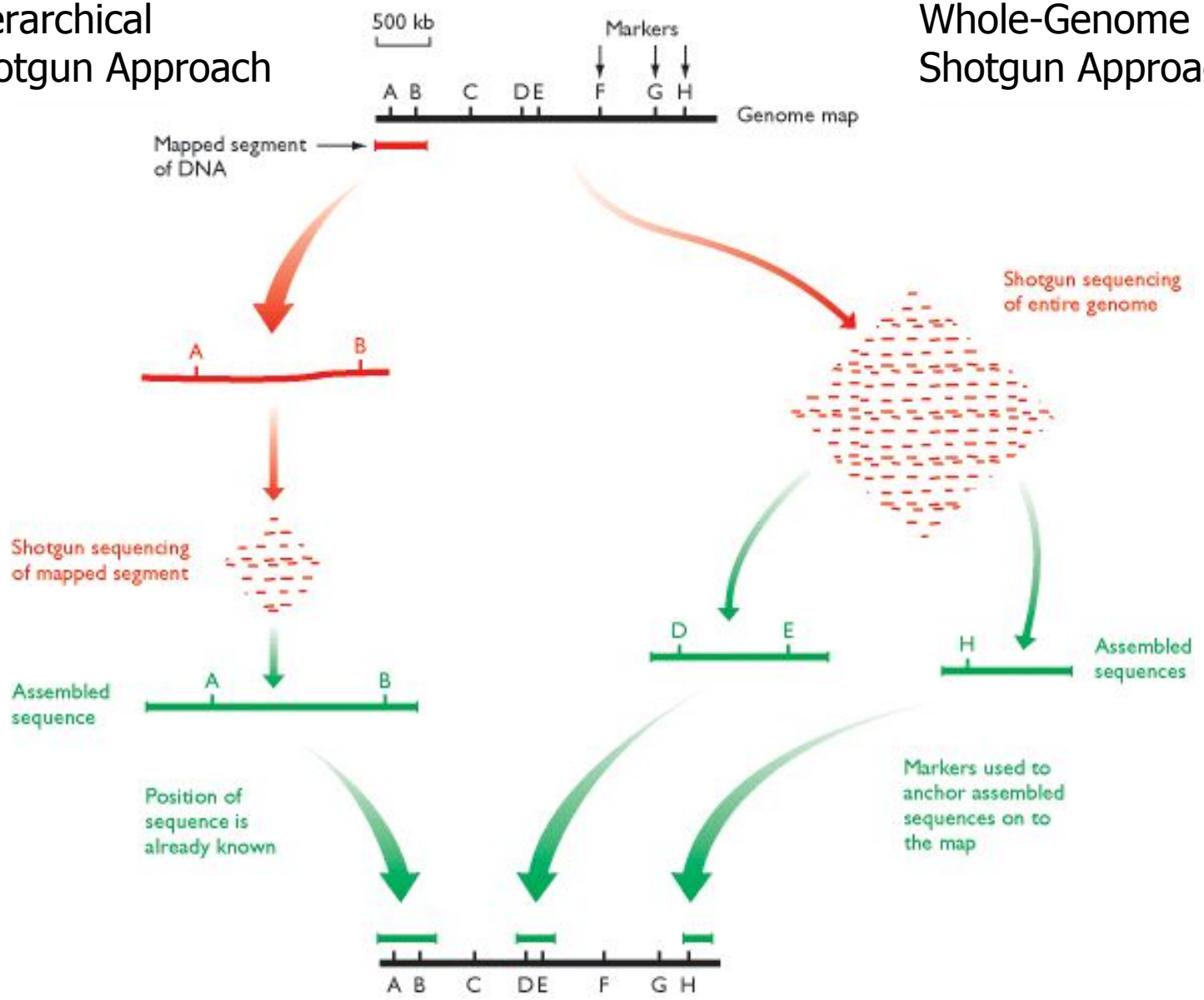
In February 2001 Celera Genomics published their draft of the human genome in the journal *Science*

The same month HUGO published its draft of the human genome in the journal *Nature*

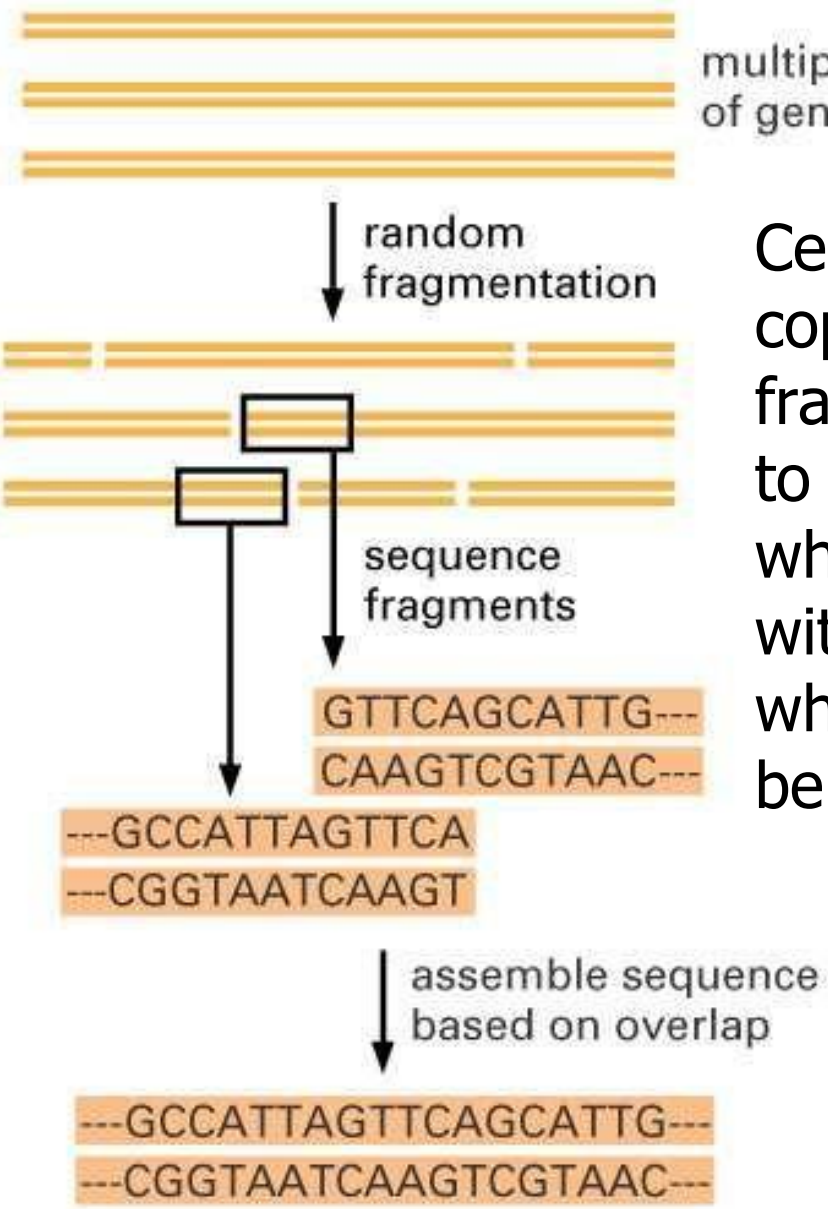
The rivals initially agreed to pool their data, but the agreement fell apart when Celera refused to deposit its data in the unrestricted public database GeneBank.

Hierarchical Shotgun Approach

Whole-Genome Shotgun Approach



http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=genomes.figgrp.6211



Celera took multiple copies of the genome fragmented them into 1 to 2kb fragments which where sequenced without concern for what chromosome they belonged to

Figure 10-9 Essential Cell Biology, 2/e. (© 2004 Garland Science)

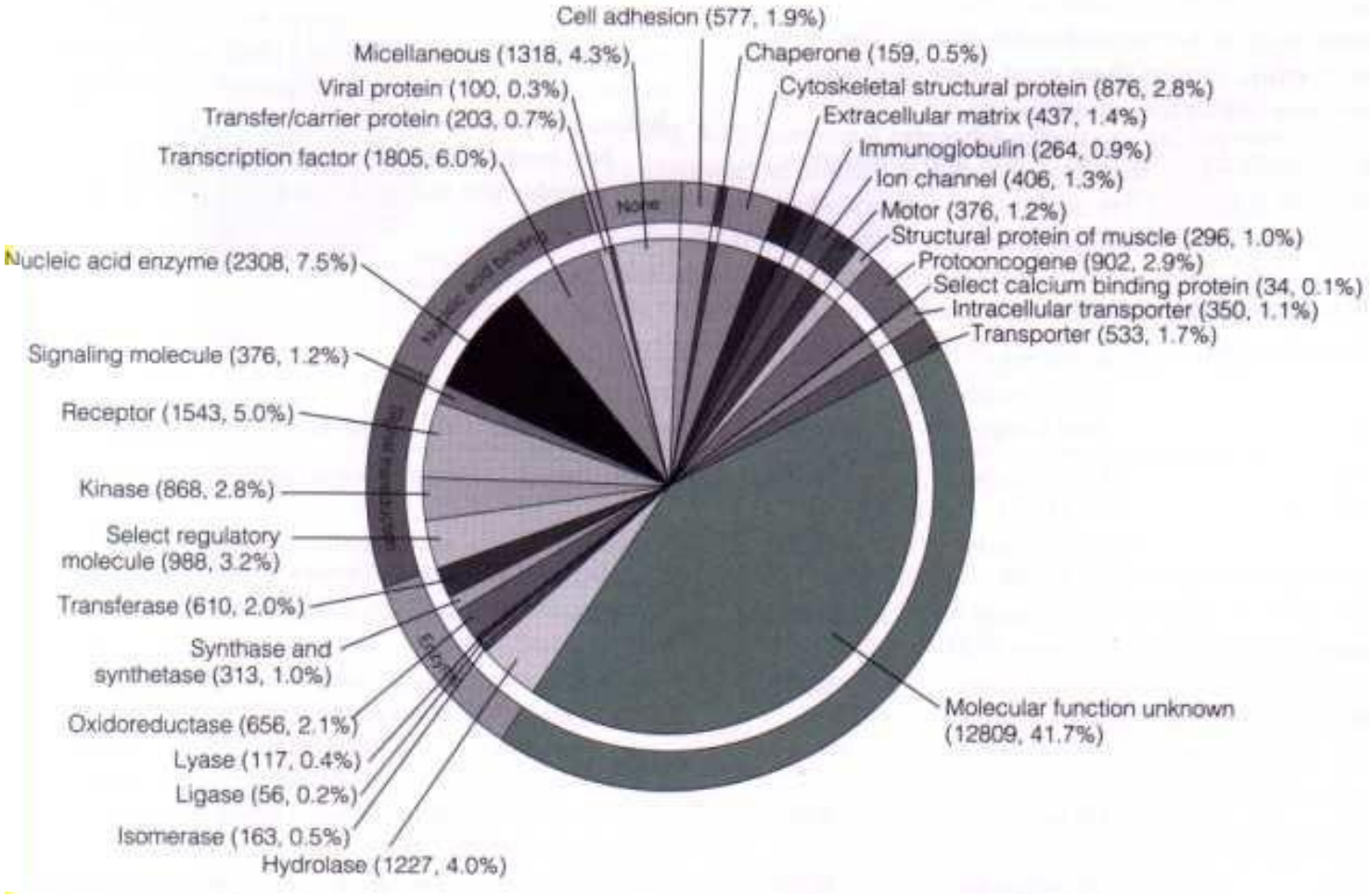
What did they learn?

1.1% of the genome is spanned by exons

24% is in introns

75% of the genome is intergenic DNA

A random pair of human haploid genomes differs on average at a rate of 1 bp per 1250 bp



Preliminary Functional Analysis of >26 000 genes

>12 000 (41%) have no known function

S. Barnum, 2005 *Biotechnology, An Introduction*. Brookes/Cole

Diploid Genome Sequence of an Individual Human

On September 4th, 2007, a team led by Craig Venter, published his (ovn) complete DNA sequence, unveiling the six-billion-letter genome of a single individual for the first time.

44% of known genes had one or more alterations

>0.5% variation between two haploid genomes

How Do We Differ?

Total of 4.1 million DNA variations

3.2 million single nucleotide changes

53,800 block substitutions (2 to 206bp)

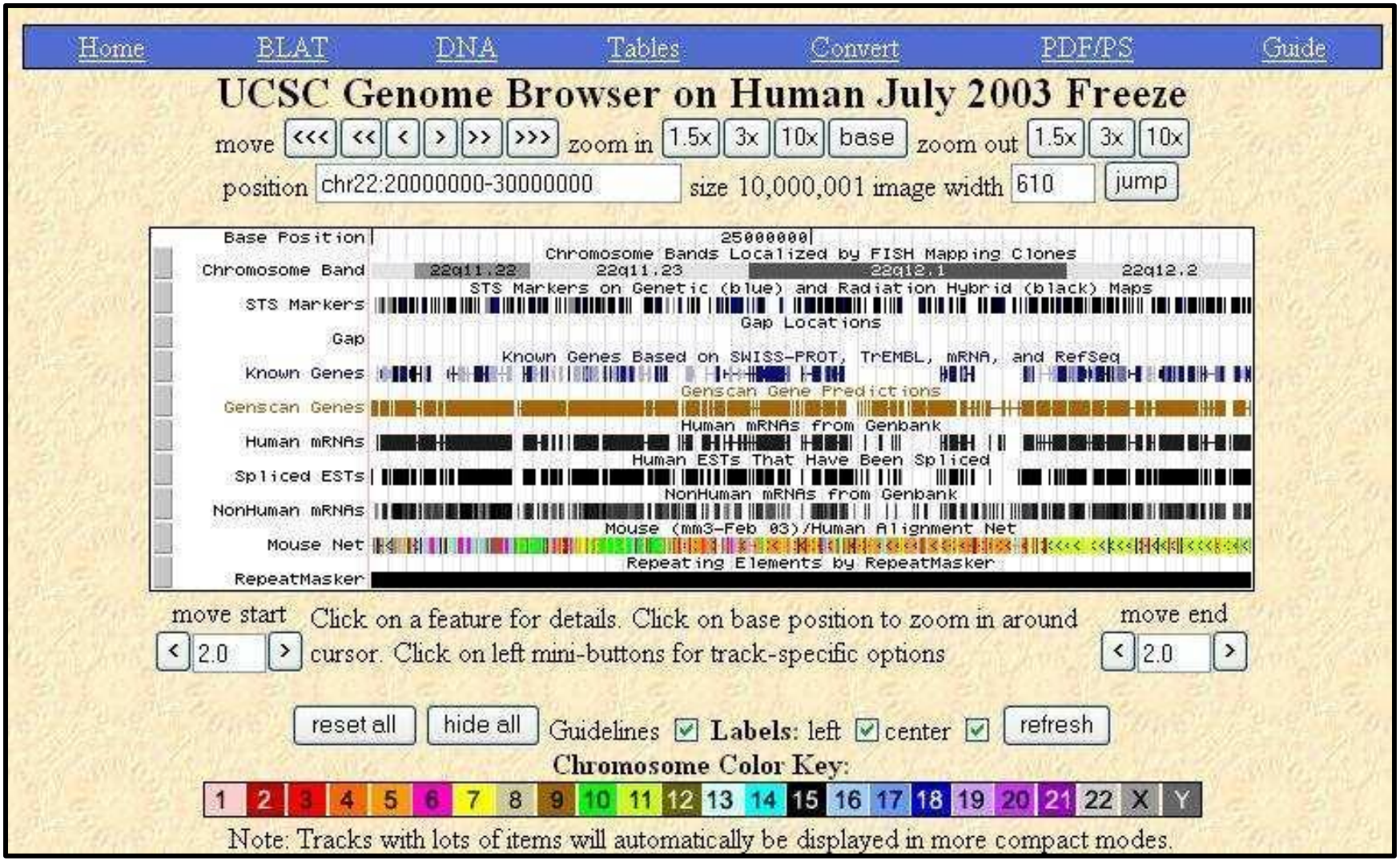
292,000 heterozygous insertion/deletions (1 to 571bp)

559,000 homozygous insertion/deletions (1 to 82,711bp)

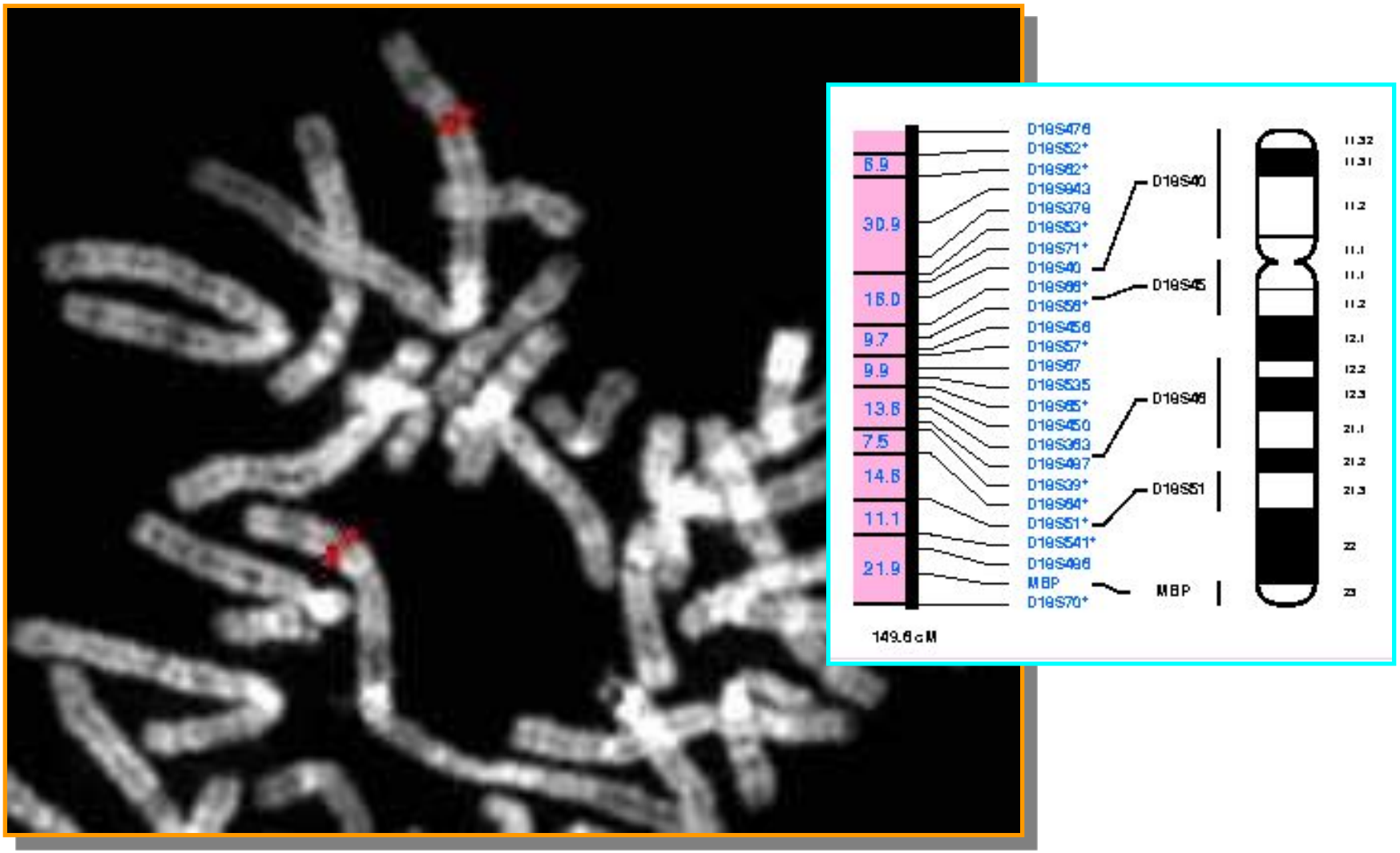
90 inversions

Numerous duplications & copy number variations

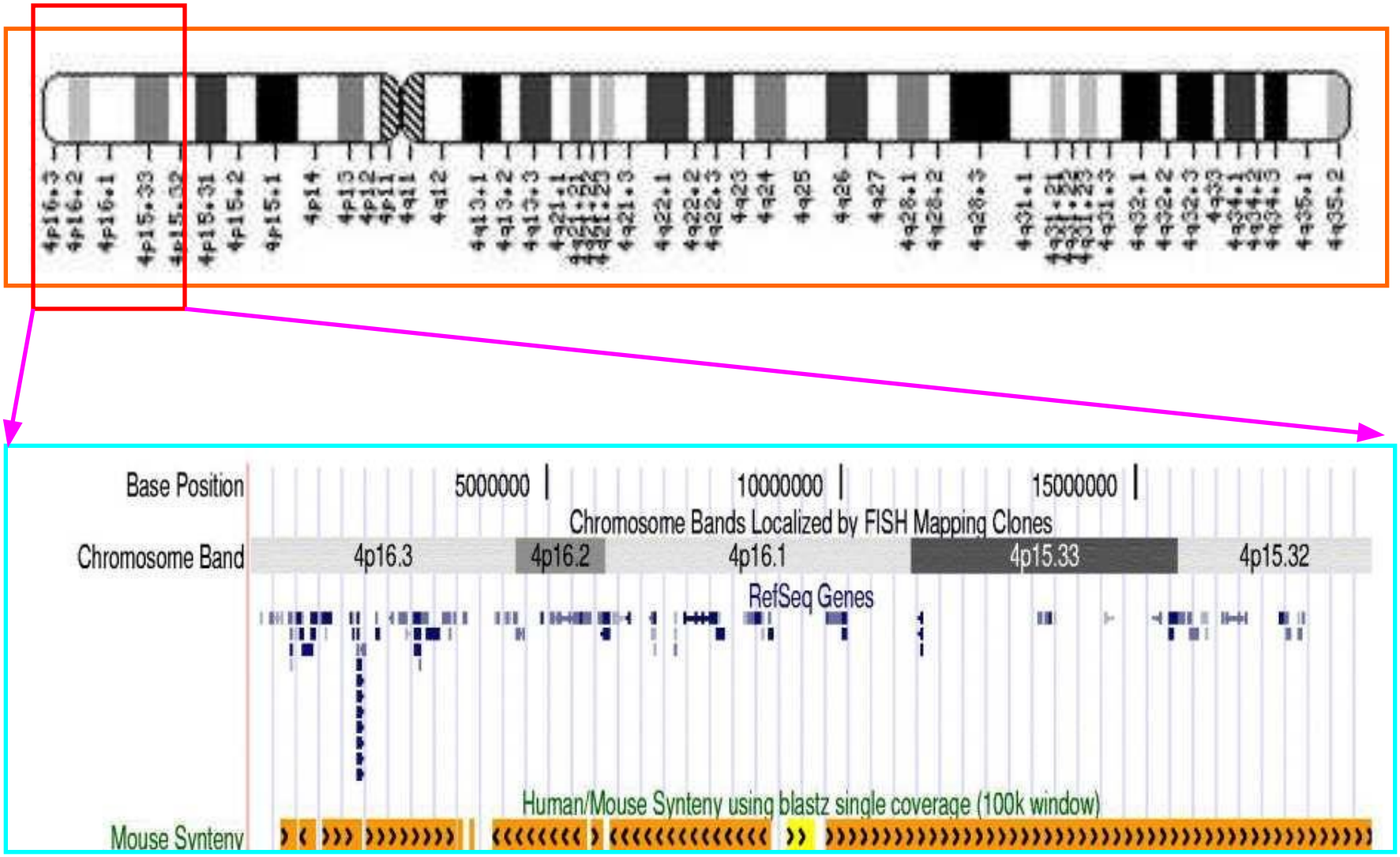
The UCSC Genome Browser



The browser takes you from early maps of the genome . . .

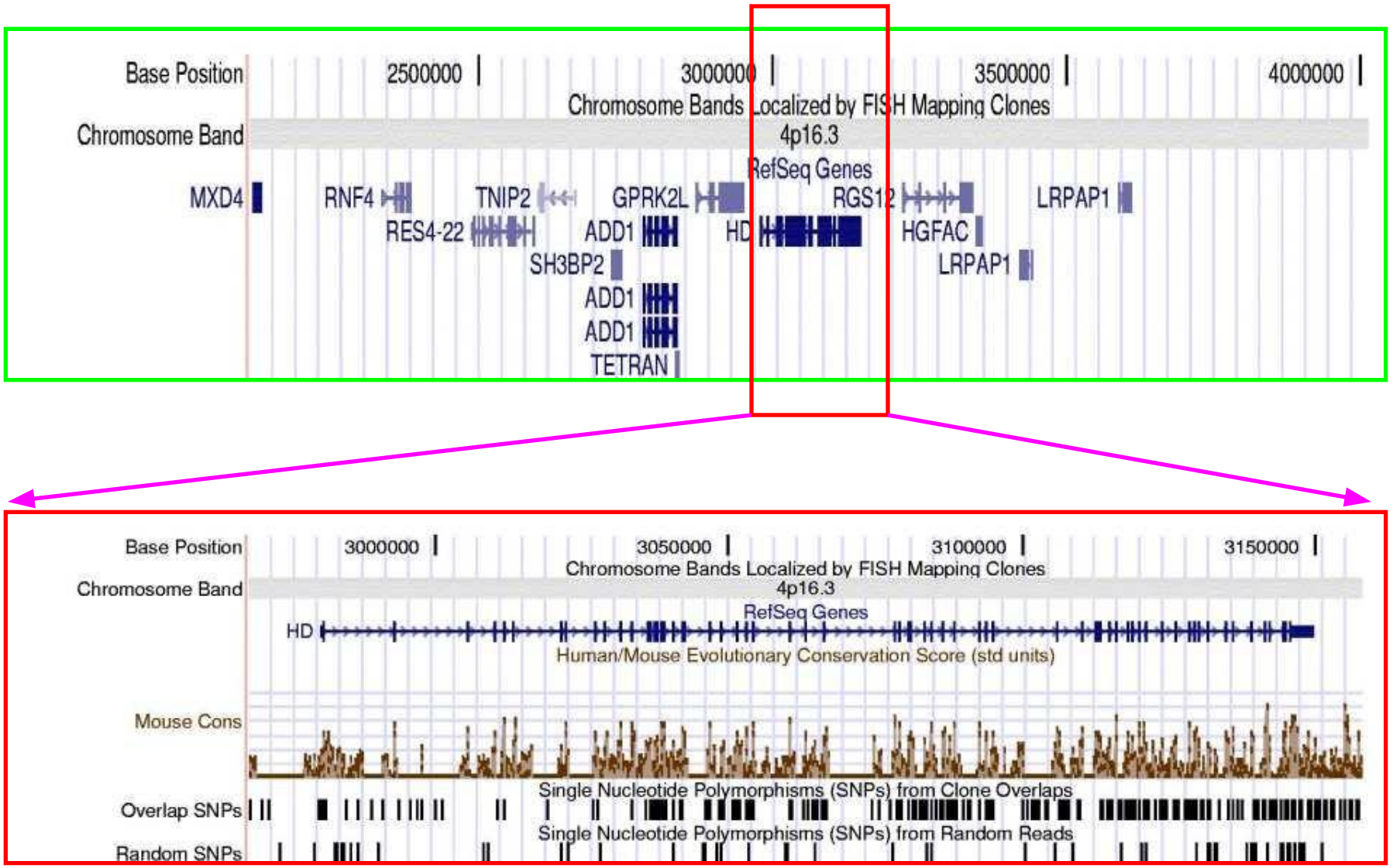


. . . to a multi-resolution view . . .

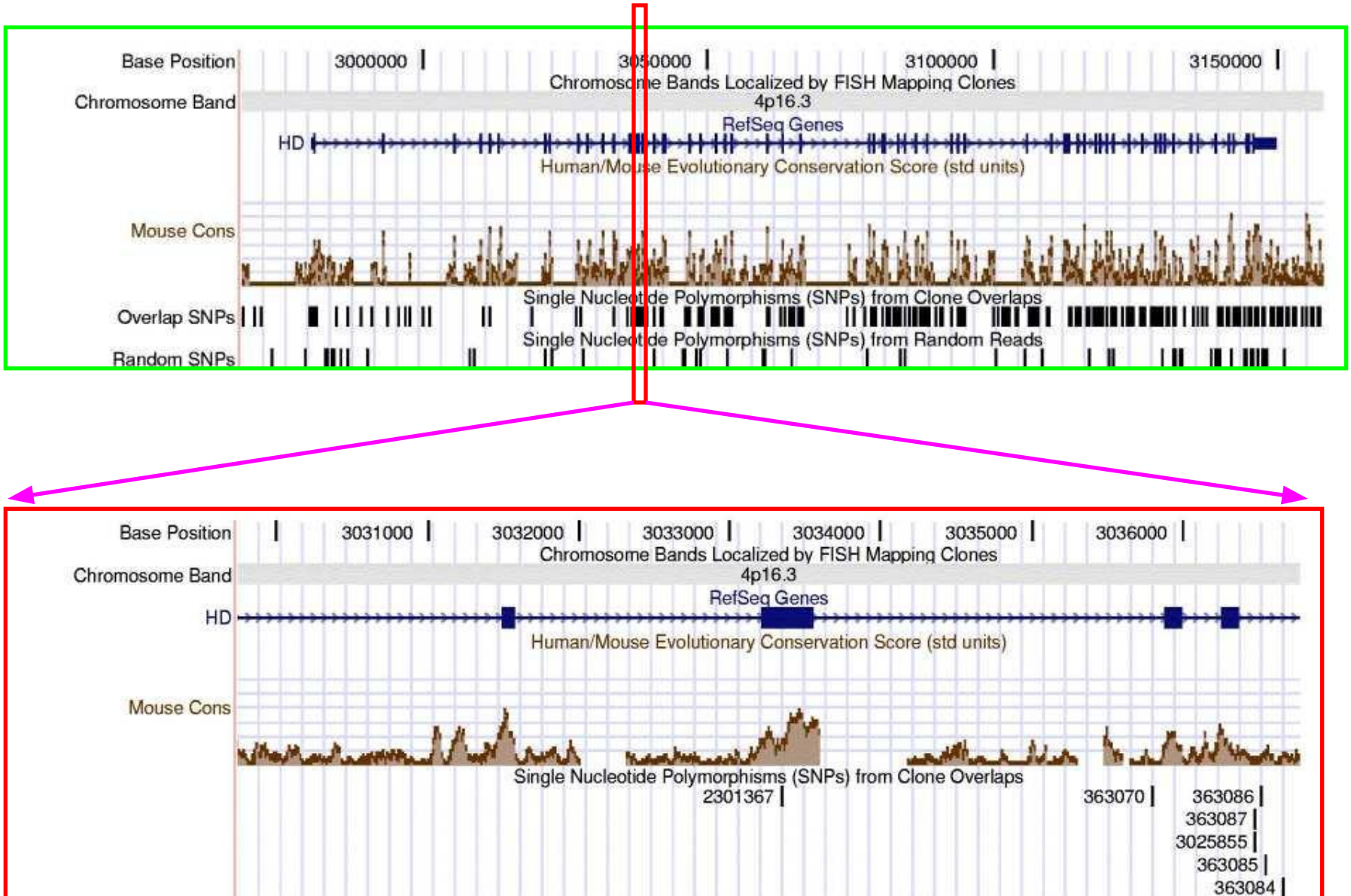


. . . at the gene cluster level . . .

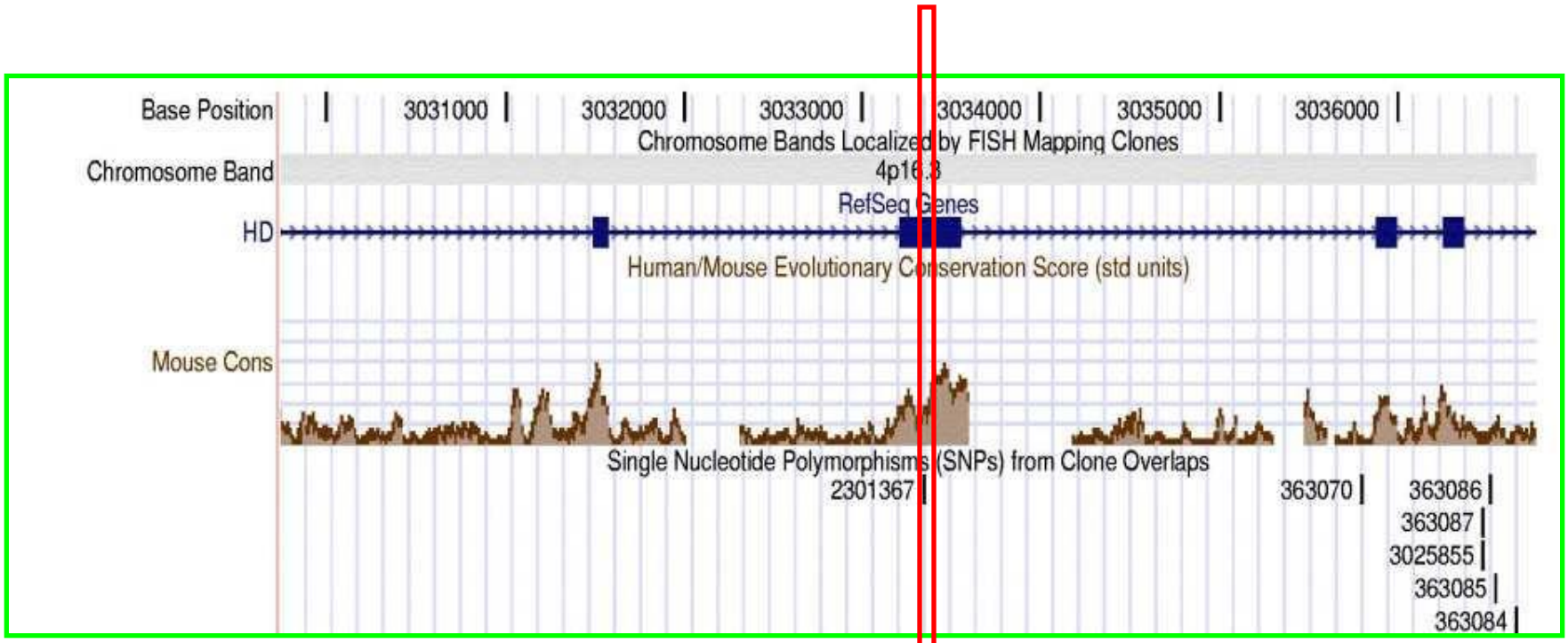
. . . the single gene level . . .



. . . the single exon level . . .



. . . and at the single base level



caggcggactcagtggatctggccagctgtgacttgacaag

caggcggactcagtggatctagccagctgtgacttgacaag

Other –omics

Proteomics

Transcriptomics

Metabolomics

Glycomics

Epigenomics

Metagenomics