HC70A Winter 2005
Professor Bob Goldberg

Lecture #8 - The Human Genome Project - Detecting & Using your Genes

Themes / Concepts

1. Genomes in Human Cells
2. Mitochondrial Genome in Medicine, Forensics, & Evolution
3. Human Genome Project - How Done?
4. Characteristics of Human Genome / Human vs. Mouse vs. Chimp — Stop 3/1/05
5. DNA Sequences in Human Genome
6. VNTRs & Utility in DNA Testing & Human Populations
7. Human Chromosomes
8. Detecting Large Changes in Human Chromosomes
9. Disease & Changes in Human Chromosome Number
10. Mutations in Human Genome - Detection & Frequency
11. SNPs - Usefulness as Genetic Markers & Measure of Diversity in Human Populations — Stop 3/8/05

2 1.5hr lectures

HUMAN GENES ARE Present
in TWO Compartments --
The Nucleus & The Mitochondria

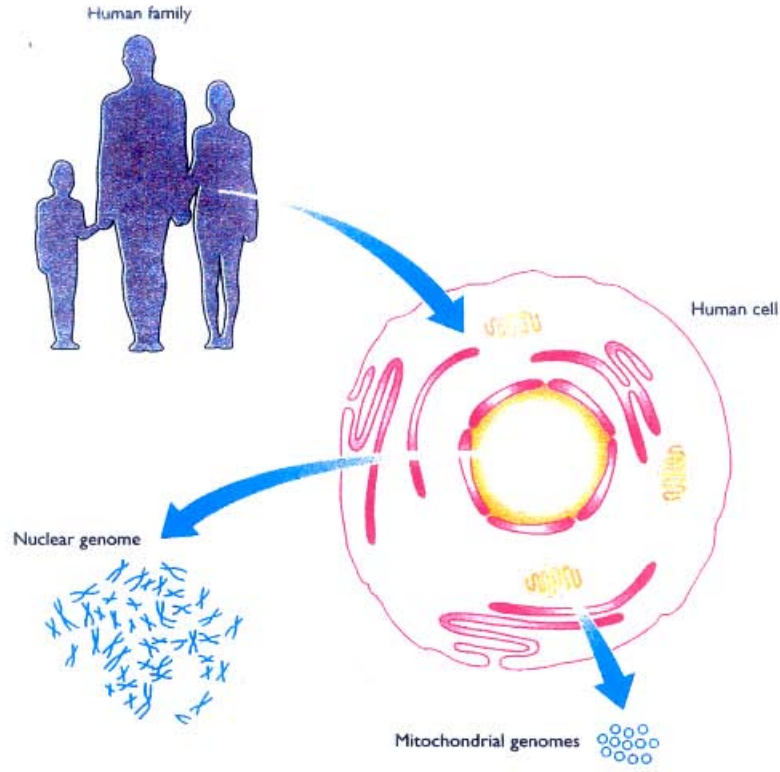
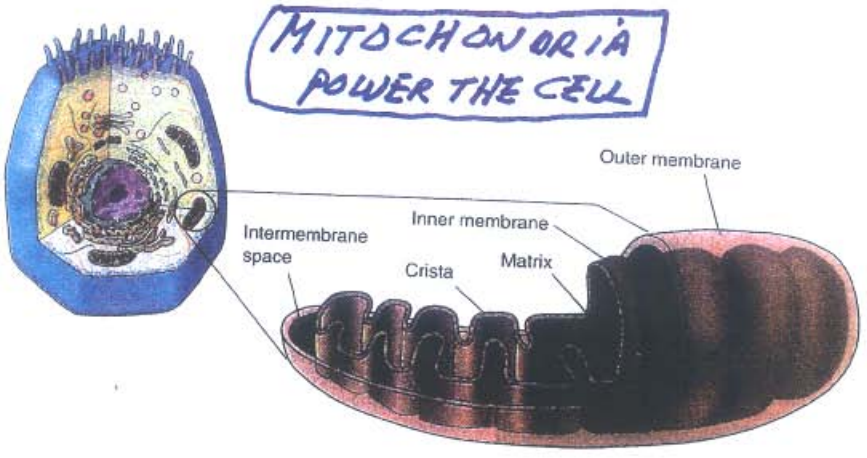


Figure 1.3 The nuclear and mitochondrial components of the human genome.
For more details on the anatomy of the human genome, see Section 6.1.

Genes in BOTH Compartments are
critical for human development.

MITOCHONDRIA POWER THE CELL



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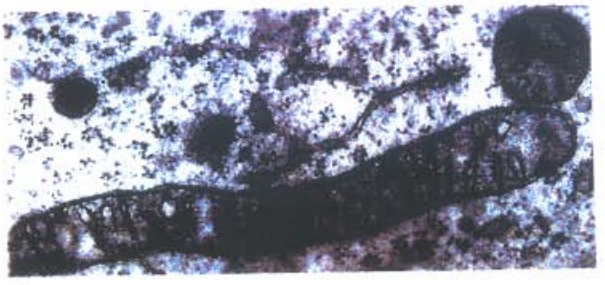


FIGURE 5.21
Mitochondria. (*a*) The inner membrane of a mitochondrion is shaped into folds called cristae, which greatly increase the surface area for oxidative metabolism. (*b*) Mitochondria in cross-section and cut lengthwise (70,000×).

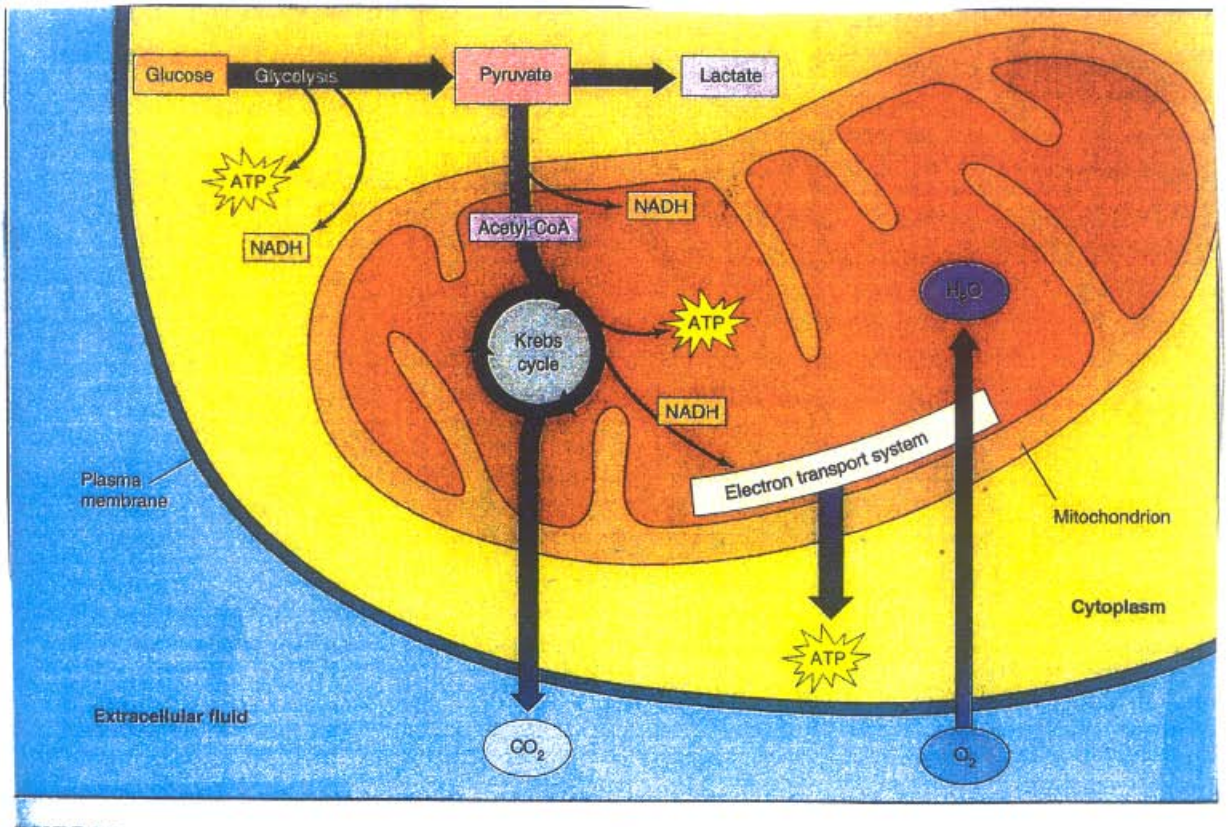


FIGURE 9.6
An overview of aerobic respiration.

(2)

MITOCHONDRIA ARE SEMI-AUTONOMOUS ORGANELLES

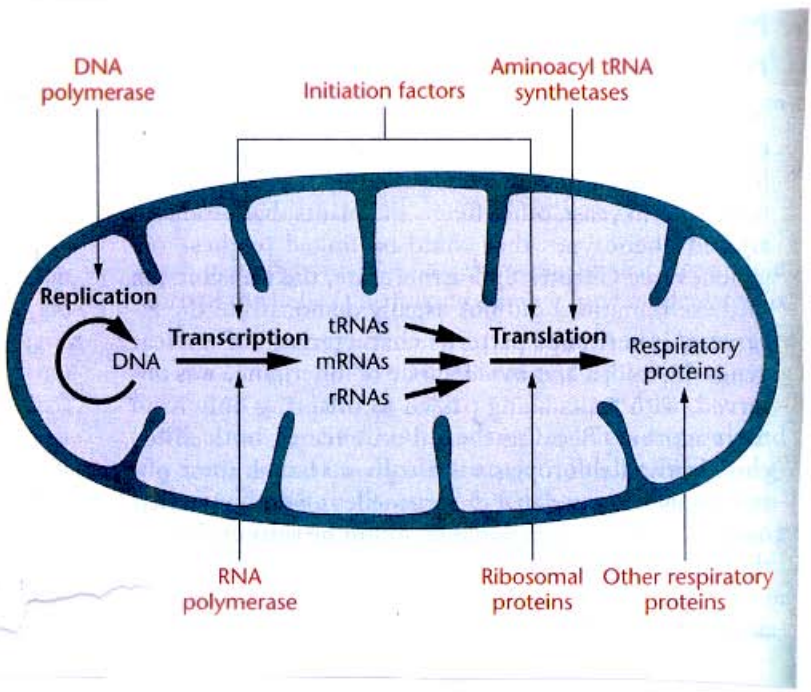


FIGURE 19.6 A comparison of the origin of gene products that are essential to mitochondrial function. Those shown entering the organelle are derived from the cytoplasm and encoded by the nucleus.

They Divide - have Genes - Encode Proteins - undergo Protein Synthesis

What are the characteristics of the Human Nuclear & Mitochondrial Genomes?

Table 7.1: The human nuclear and mitochondrial genomes

	Nuclear genome 3.3×10^9 bp	Mitochondrial genome 16,600 bp
Size	3300 Mb	16.6 kb
No. of different DNA molecules	23 (in XX) or 24 (in XY) cells, all linear	One circular DNA molecule
Total no. of DNA molecules per cell	23 in haploid cells; 46 in diploid cells	Several thousand
Associated protein	Several classes of histone and nonhistone protein	Largely free of protein
Number of genes	~65 000–80 000	37
Gene density	~1/40 kb	1/0.45 kb
Repetitive DNA	Large fraction, see *Figure 7.1.*	Very little
Transcription	The great bulk of genes are transcribed individually	Continuous transcription of multiple genes
Introns	Found in most genes	Absent
% of coding DNA	~3%	~93%
Codon usage	See *Figure 1.22*	See *Figure 1.22*
Recombination	At least once for each pair of homologs at meiosis	Not evident
Inheritance	Mendelian for sequences on X and autosomes; paternal for sequences on Y	Exclusively maternal

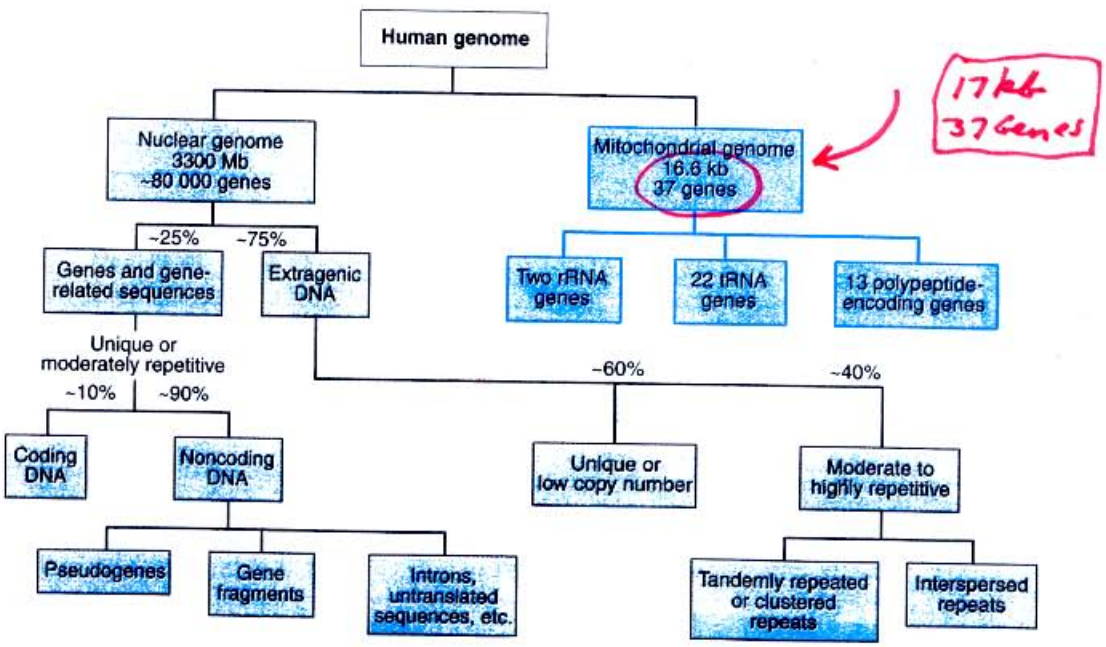


Figure 7.1: Organization of the human genome.

Features of the Human Nuclear and Mitochondrial Genomes

Table 9.1: The human nuclear and mitochondrial genomes

	Nuclear genome	Mitochondrial genome
Size	3200 Mb	16.6 kb
No. of different DNA molecules	23 (in XX cells) or 24 (in XY cells); all linear	One circular DNA molecule
Total no. of DNA molecules per cell	46 in diploid cells, but varies according to ploidy	Often several thousands (but variable – see *Box 9.1*)
Associated protein	Several classes of histone and nonhistone protein	Largely free of protein
No. of genes	~ 30 000–35 000	37
Gene density	~ 1/100 kb	1/0.45 kb
Repetitive DNA	Over 50% of genome, see *Figure 9.1*	Very little
Transcription	The great bulk of genes are transcribed individually (*monocistronic transcription units*)	Co-transcription of multiple genes from both the heavy and the light strands (*polycistronic transcription units*)
Introns	Found in most genes	Absent
% of coding DNA	~ 1.5%	~ 93%
Codon usage	See *Figure 1.22*	See *Figure 1.22*
Recombination	At least once for each pair of homologs at meiosis	Not evident
Inheritance	Mendelian for sequences on X and autosomes; paternal for sequences on Y	Exclusively maternal

The Mitochondrial Genome is A SMALL CIRCLE Containing only 37 Genes

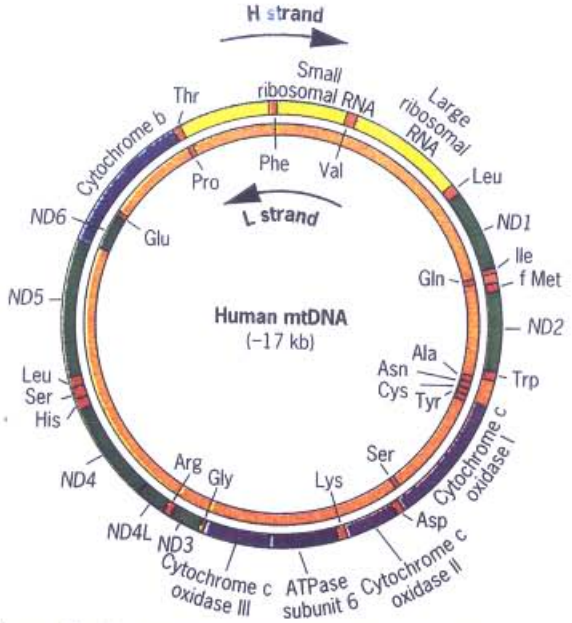
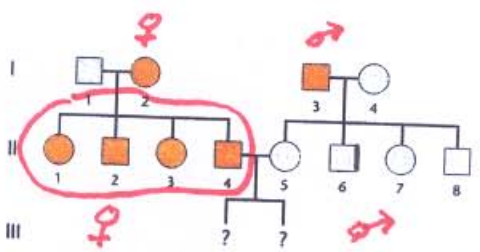


Figure 19.14 Map of human mtDNA showing the pattern of transcription. Genes on the inner circle are transcribed from the L strand of the DNA, whereas genes on the outer circle are transcribed from the H strand of the DNA. Arrows show the direction of transcription. *ND1–6* are genes encoding subunits of the enzyme NADH reductase; the tRNA genes in the mtDNA are indicated by abbreviations for the amino acids.

MtDNA is a CIRCLE

Mitochondrial Genes ARE Inherited MATERNALLY



PASSED DIRECTLY FROM Mother to Children

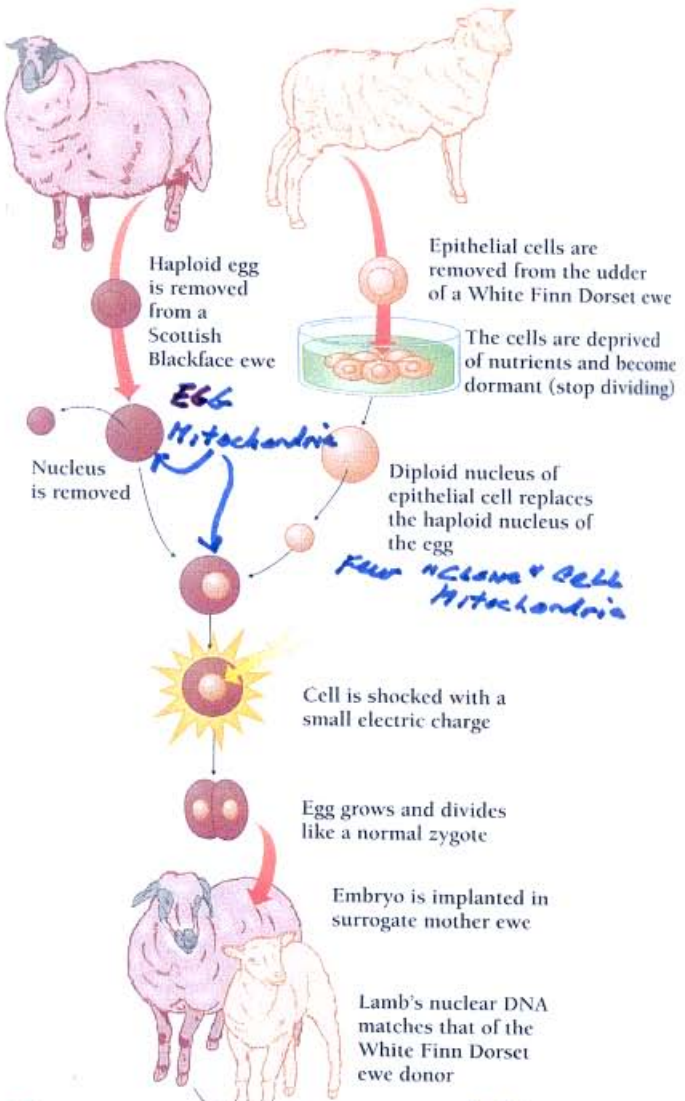
Hypothesis to Explain?

↳ all Children

↳ no Children

(5)

IN A CLONING EXPERIMENT MOST OF MITOCHONDRIA COMES FROM EGG DONOR!



∴ CLONE NOT TECHNICALLY A CLONE WITH RESPECT TO MITOCHONDRIAL GENES

WILL BE MOSAIC - MOST MT FROM EGG DONOR - FEW MT FROM CELL USED FOR CLONE'S GENOME!!

CLONE'S NUCLEAR GENOME / EGG DONOR MT GENOME!

Figure 44-16 Cloning Dolly. The trick to cloning Dolly was to make differentiated cells less differentiated. By depriving the cultured udder cells of nutrients, the researchers induced the nuclei to enter a dormant state.

ANOTHER POTENTIAL PROBLEM SOURCE FOR CLONE DEVELOPMENT!

Several Mitochondrial Diseases occur in Humans

In order for a human disorder to be attributable to genetically altered mitochondria, several criteria must be met.

1. Inheritance must exhibit a maternal rather than a Mendelian pattern.
2. The disorder must reflect a deficiency in the bioenergetic function of the organelle.
3. There must be a specific genetic mutation in one of the mitochondrial genes.

Thus far, several cases are known to demonstrate these characteristics. For example, **myoclonic epilepsy and ragged red fiber disease (MERRF)** demonstrates a pattern of inheritance consistent with maternal inheritance. Only offspring of affected mothers inherit the disorder; the offspring of affected fathers are all normal. Individuals with this rare disorder express deafness, dementia, and seizures. Both muscle fibers and mitochondria are affected; the aberrant mitochondria characterize what are described as ragged red fibers (**RRFs**) of skeletal muscle (Figure 8.5). Analysis of mtDNA has revealed a mutation in one of the mitochondrial genes encoding a transfer RNA. This genetic alteration apparently interferes with translation within the organelle, which in turn leads to the various manifestations of the disorder.

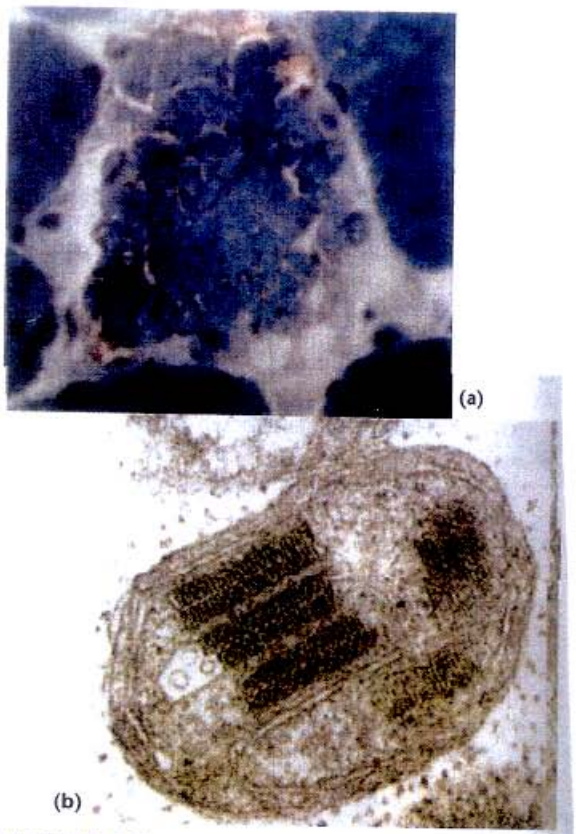


FIGURE 8.5 Mitochondrial myopathy in skeletal muscle cells of a patient with MERFF. Part (a) shows a ragged red fiber with abnormal mitochondria. Part (b) shows an abnormal mitochondrion revealing paracrystalline arrays within it.

A second disorder, **Leber's hereditary optic neuropathy (LHON)**, also exhibits maternal inheritance as well as mtDNA lesions. The disorder is characterized by sudden bilateral blindness. The average age of vision loss is 27, but onset is quite variable. Four mutations have been identified, all of which disrupt normal oxidative phosphorylation. Over 50 percent of cases are due to a mutation at a specific position in the mitochondrial gene encoding a subunit of NADH dehydrogenase so that the amino acid arginine is converted to histidine. This mutation is transmitted to all maternal offspring. It is interesting to note that in many instances of LHON, there is no family history; a significant number of cases appear to result from "new" mutations.

Individuals severely affected by a third disorder, **Kearns-Sayre syndrome (KSS)**, lose their vision, undergo hearing loss, and display heart conditions. The genetic basis of KSS involves deletions at various positions within mtDNA. Many KSS patients are symptom-free as children but display progressive symptoms as adults. The proportion of mtDNAs that reveal deletions increases as the severity of symptoms increases.

The study of hereditary mitochondrial-based disorders provides insights into the importance and genetic basis of this organelle during normal development, as well as the relationship between mitochondrial function and neuromuscular disorders. Such study has also suggested a hypothesis for aging based on the progressive accumulation of mtDNA mutations and the accompanying loss of mitochondrial function.

Mitochondrial Mutations LEADING TO DISEASES

Table 16.1 Phenotypes associated with some mitochondrial mutations

Nucleotide changed	Mitochondrial component affected	Phenotype[a]
3460	ND1 of Complex I[b]	LHON
11778	ND4 of Complex I	LHON
14484	ND6 of Complex I	LHON
8993	ATP6 of Complex V[b]	NARP
3243	$tRNA^{Leu(UUR)}$[c]	MELAS, PEO
3271	$tRNA^{Leu(UUR)}$	MELAS
3291	$tRNA^{Leu(UUR)}$	MELAS
3251	$tRNA^{Leu(UUR)}$	PEO
3256	$tRNA^{Leu(UUR)}$	PEO
5692	$tRNA^{Asn}$	PEO
5703	$tRNA^{Asn}$	PEO, myopathy
5814	$tRNA^{Cys}$	Encephalopathy
8344	$tRNA^{Lys}$	MERRF
8356	$tRNA^{Lys}$	MERRF
9997	$tRNA^{Gly}$	Cardiomyopathy
10006	$tRNA^{Gly}$	PEO
12246	$tRNA^{Ser(AGY)}$[c]	PEO
14709	$tRNA^{Glu}$	Myopathy
15923	$tRNA^{Thr}$	Fatal infantile multisystem disorder
15990	$tRNA^{Pro}$	Myopathy

[a]LHON Leber's hereditary optic neuropathy; NARP Neurogenic muscle weakness, ataxia, retinitis pigmentosa; MERRF Myoclonic epilepsy and ragged-red fiber syndrome; MELAS Mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes; PEO Progressive external ophthalmoplegia

[b]Complex I is NADH dehydrogenase. Complex V is ATP synthase.

[c]In $tRNA^{Leu(UUR)}$, the R stands for either A or G; in $tRNA^{Ser(AGY)}$, the Y stands for either T or C.

www.mitomap.org

MAP OF MITOCHONDRIAL GENES AND CORRESPONDING DISEASES

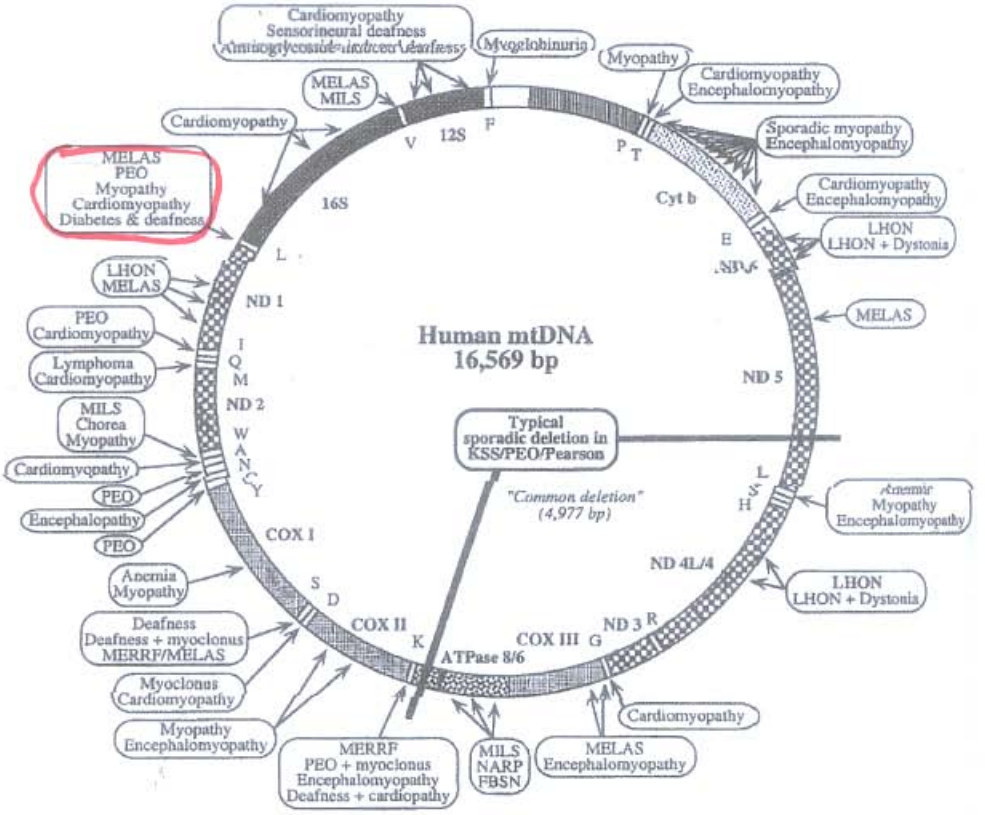


FIGURE 18.16. Morbidity map of the human mitochondrial genome. Abbreviations are for the genes encoding seven subunits of complex I (ND), three subunits of cytochrome c oxidase (COX), cytochrome b (Cyt b), and the two subunits of ATP synthase (ATPase 6 and 8). 12S and 16S refer to ribosomal RNAs; 22 transfer RNAs are identified by the one-letter codes for the corresponding amino acids. FBSN, familial bilateral striatal necrosis; KSS, Kearns–Sayre syndrome; LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes; MERRF, myoclonic epilepsy with ragged-red fibers; MILS, maternally inherited Leigh syndrome; NARP, neuropathy, ataxia, retinitis pigmentosa; PEO, progressive external ophthalmoplegia. From DiMauro and Schon (2001). Used with permission.

Mitochondrial Diseases ARE Inherited Maternally

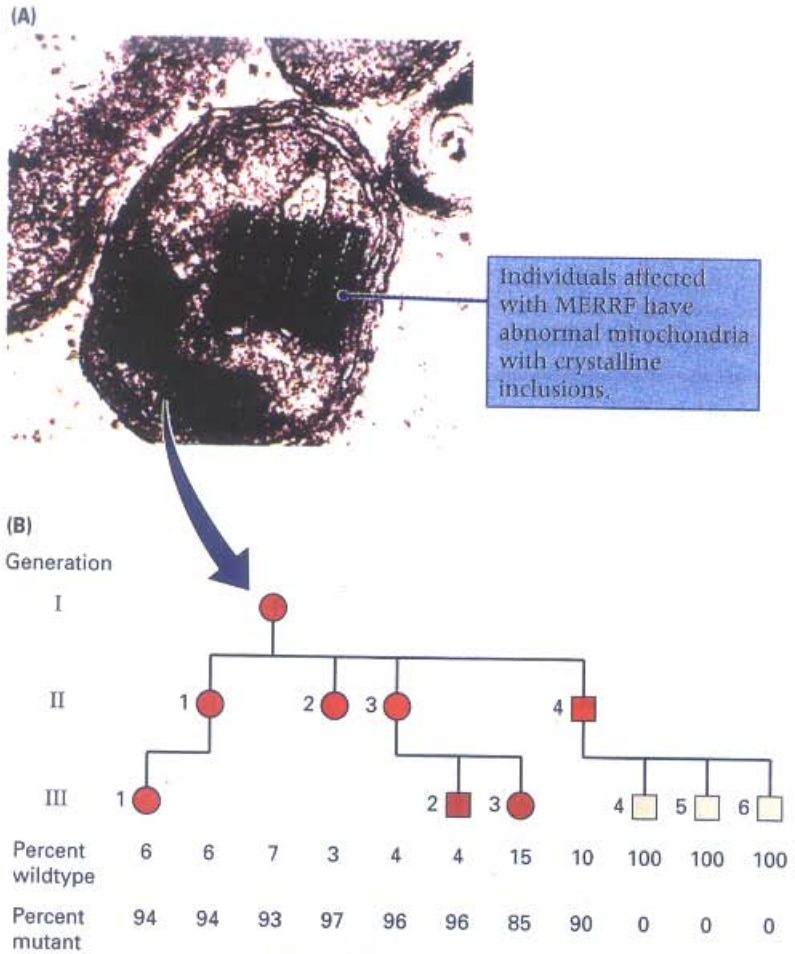


Figure 16.2 Inheritance of myoclonic epilepsy with ragged-red fiber disease (MERRF) in humans. (A) Electron micrograph of an abnormal MERRF mitochondrion containing paracrystalline inclusions. (B)The pedigree shows inheritance of MERRF in one family and the percentage of the mitochondria in each person found to be wildtype or mutant. [Micrograph courtesy of D. C. Wallace, from J. M. Shoffner, M. T. Lott, A. M. S. Lezza, P. Seibel, S. W. Ballinger, and D. C. Wallace. 1990. *Cell* 61: 931.]

Never passed on from diseased ♂ to Children

Mitochondria absent from sperm "Head"

Mitochondrial RFLP Markers can be used to Follow Diseased Mt Genes

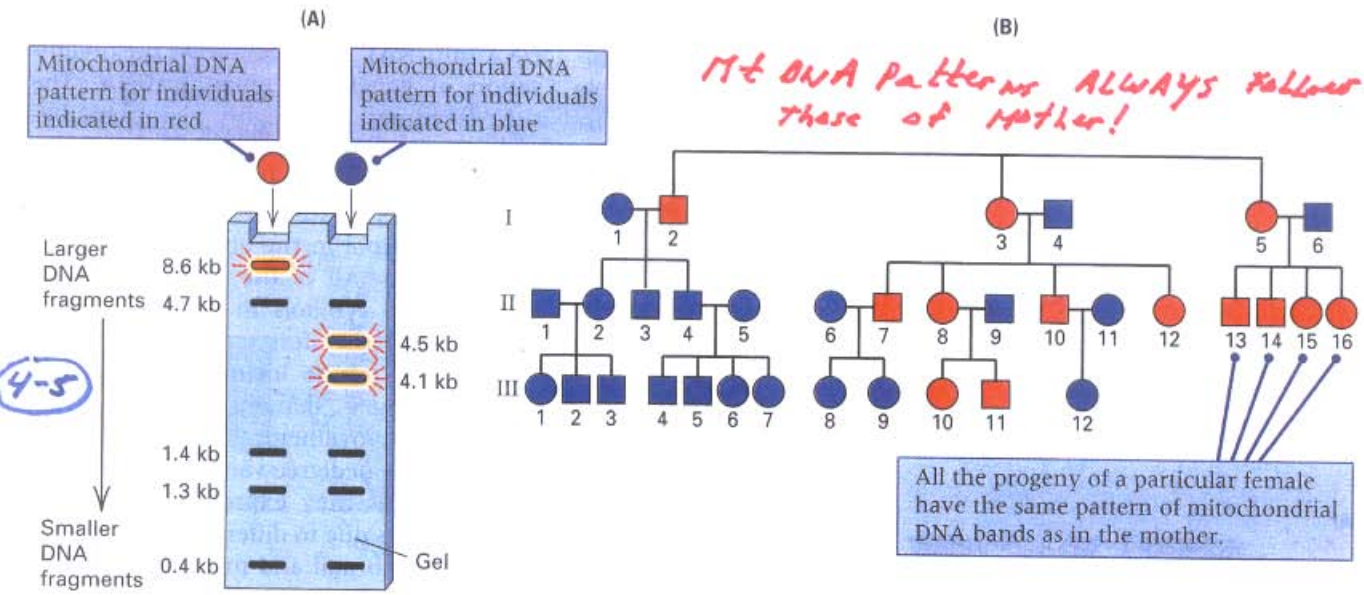


Figure 16.1 Maternal inheritance of human mitochondrial DNA. (A) Pattern of DNA fragments obtained when mitochondrial DNA is digested with the restriction enzyme *Hae*II. The DNA type at the left includes a fragment of 8.6 kb (red). The DNA type at the right contains a cleavage site for *Hae*II within the 8.6-kb fragment, which results in smaller fragments of 4.5 kb and 4.1 kb (blue). (B) Pedigree showing maternal inheritance of the DNA pattern with the 8.6-kb fragment (red symbols). The mitochondrial DNA type is transmitted only through the mother. [After D. C. Wallace. 1989. *Trends in Genetics* 5: 9.]

Because Mt Genome is SMALL (16kb) — Restriction fragments can be seen directly in Gel — No BLOT needed

How Many EcoRI Fragments in Mt Genome with 50% G+C ?

Maternal Inheritance of Mitochondrial DNA

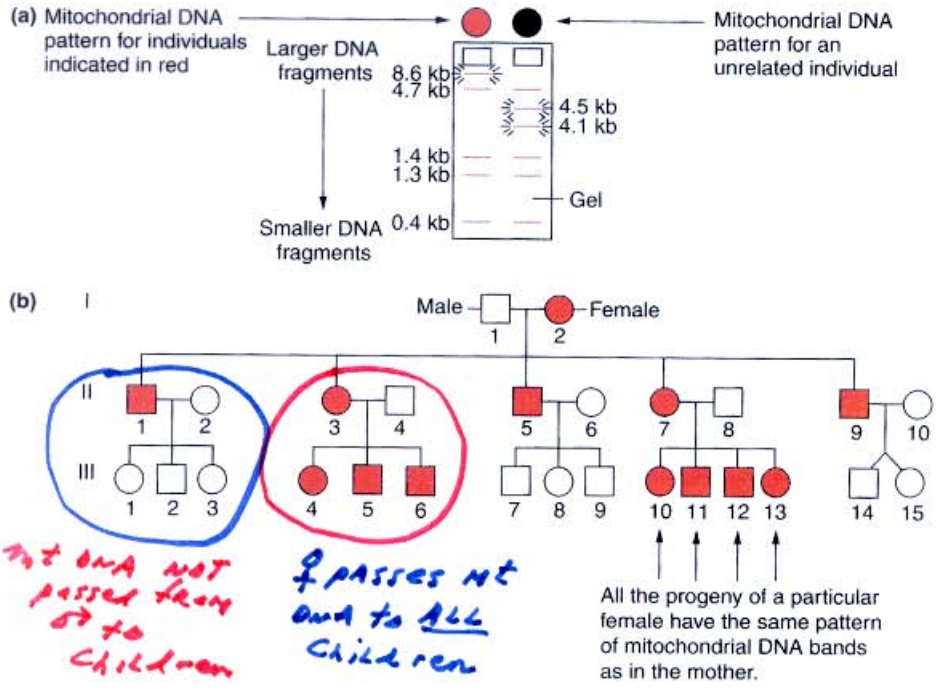
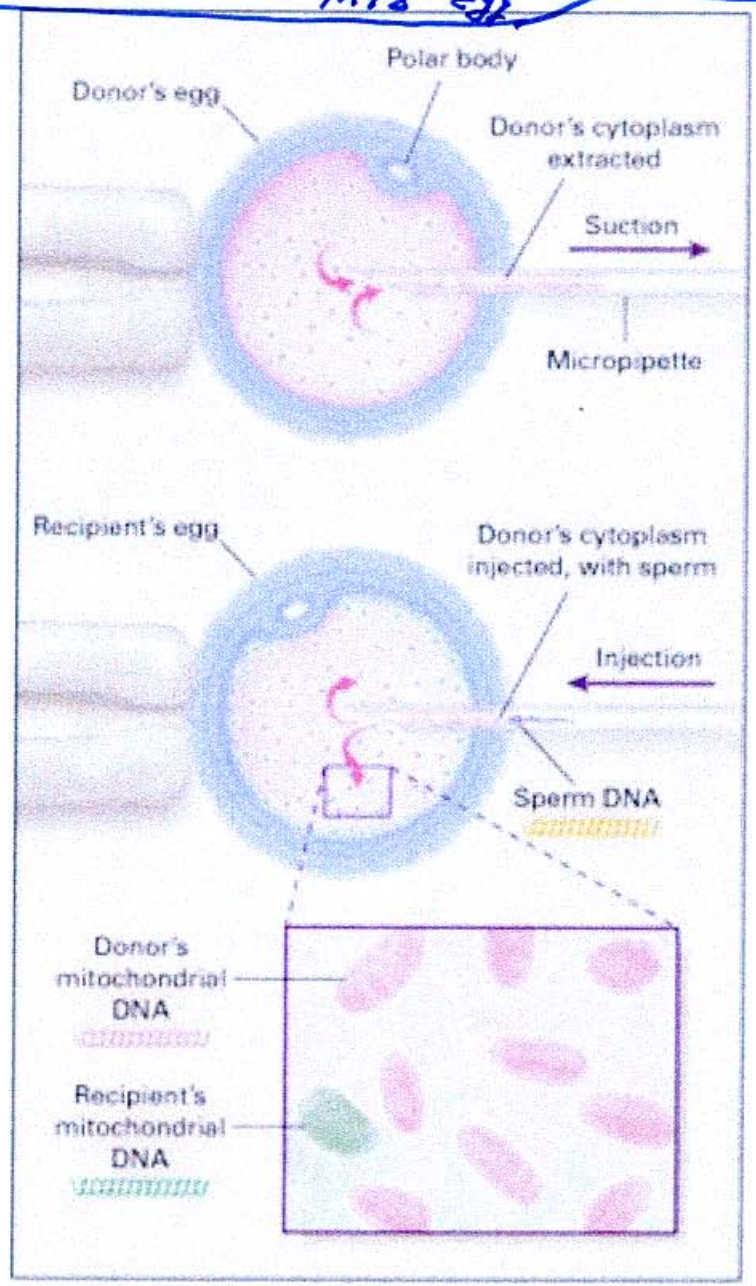


Figure 8.11 Maternal DNA Pattern of Inheritance Key genes for cell respiration and mitochondrial function are located in a small DNA ring in human mitochondria. Because mitochondria are contributed by the egg before fertilization, DNA can be traced through the maternal line with fingerprinting of the mitochondrial DNA.

Mt DNA NOT passed from ♂ to Children

♀ passes Mt DNA to ALL Children

OOPLASMIC TRANSFER TO INJECT HEALTHY Mitochondrial Genomes into Egg

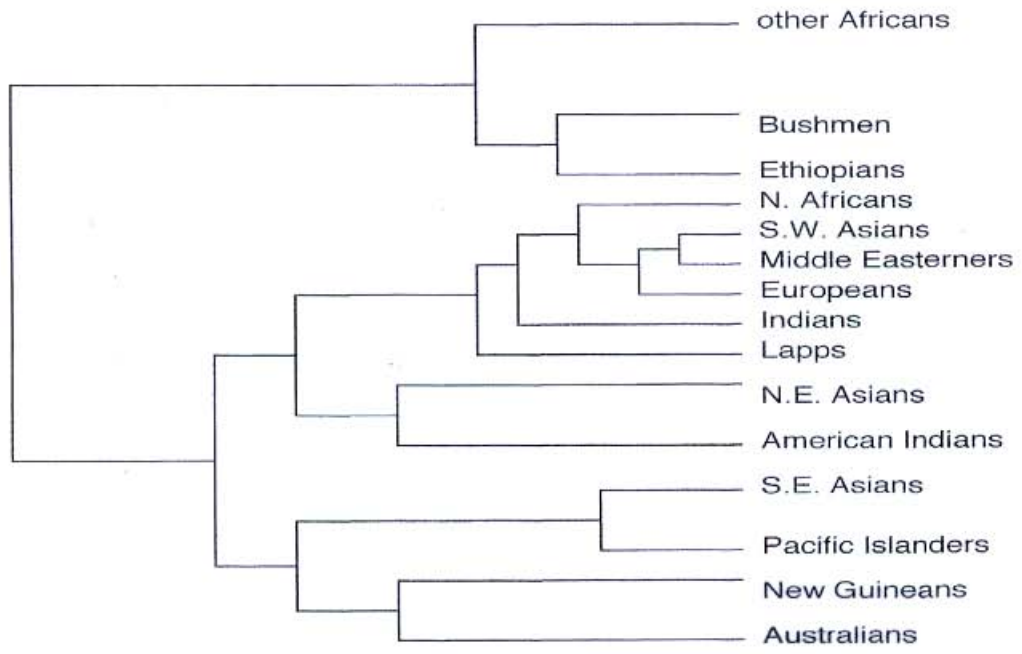


FORM OF Gene Therapy That's Inherited

911

Using Mt DNA Polymorphisms to construct trees of Human origins

Evolutionary Tree



Passed on FROM "Eve" to us!

MITOCHONDRIAL FINGERPRINTS / POLYMORPHISMS CAN BE USED TO STUDY HUMAN ORIGINS OR FIND THE FIRST "EVE"

HUMAN GENETICS SIDELIGHT

Using Mitochondrial DNA to Study Human Evolution

In biology few subjects are more fascinating than that of human evolution. Who are we? Where did we come from? Where are we going? Before the advent of molecular biology, the study of human evolution depended on the analysis of rare fossils—fragments of bone, a few teeth, an occasional weapon or tool. Today, human evolution can be studied by comparing DNA sequences. Each DNA sequence is descended from a sequence that was present in an ancestral organism. Thus, the DNA sequences that we find today are, in effect, living fossils—records of ancient DNA that has been transmitted through many generations to organisms currently alive. Because mutations may have occurred during this time, a modern DNA sequence is not likely to be an exact replica of its ancestor. However, by comparing modern DNA sequences, we can sometimes reconstruct features of the evolutionary process that produced them.

Some of the most insightful studies of human evolution have involved the analysis of mitochondrial DNA. There are two reasons why mtDNA is so useful: (1) it evolves faster than nuclear DNA, and (2) it is transmitted exclusively through the female. The rapidity of mtDNA evolution allows a scientist to detect significant genetic changes over a relatively short period of time (in evolutionary terms), and the strict maternal transmission of mtDNA allows a researcher to trace modern DNA sequences back to a common female ancestor.

Pioneering studies of human mtDNA were carried out in the 1980s by Allan Wilson, Rebecca Cann, Mark Stoneking, and their colleagues. These studies established that there is relatively little variation in the mtDNA from different human populations and that the greatest variation is found in the mtDNA from populations in Africa. Given the rate at which mtDNA is known to evolve, these discoveries suggested that modern human beings originated rather recently, probably within the last 200,000 years, and probably in Africa. Although these conclusions were initially controversial, later work has reinforced them.[1] Wilson's laboratory collected mtDNA samples from more than 200 individuals representing many different racial and ethnic groups. The mtDNA sequences in this collection were determined biochemically and then analyzed by a computer program that arranges the sequences in a phylogenetic, or evolutionary, tree. Wilson's conclusion was startling. The mtDNA in all modern groups of humans is descended from an mtDNA molecule that existed in a single woman who lived in Africa about 200,000 years ago. Applying a biblical metaphor, the popular press nicknamed this woman "Mitochondrial Eve."

By focusing on the evolution of mtDNA, Wilson's laboratory traced human ancestry back to a point where the maternal lineages of all modern mtDNA sequences coalesce in a single common ancestor—the mitochondrial mother of us all. However, these researchers never meant to imply that a single woman alone gave rise to all modern human beings. The mass of human nuclear DNA, which is inherited equally from males and females, and which varies among the members of a breeding population, cannot be traced to a single individual.

The work of Wilson and his colleagues strongly argues that all modern humans evolved from individuals who lived in Africa less than 200,000 years ago, and possibly as recently as 120,000 years ago. Migrants from this original African population presumably founded the archaic human populations of Europe and Asia, which, in turn, founded the early human populations of Australia, Oceana, and the Americas. This evolutionary scenario has been called the "Out of Africa" hypothesis. Another hypothesis proposes that humans evolved simultaneously in many regions of the world, from groups that were long established in those regions, perhaps for many hundreds of thousands of years, and that these groups probably interbred with other archaic populations such as the Neanderthals of Europe and western Asia.

The Neanderthals have always been an enigmatic group for students of human evolution. Fossil remains indicate that they were quite different from modern humans; thicker bones, greater musculature, and different body proportions clearly set them apart. Were the Neanderthals ancestral to modern humans? Did they interbreed with the populations that ultimately produced modern humans, or were they a separate and distinct species altogether?

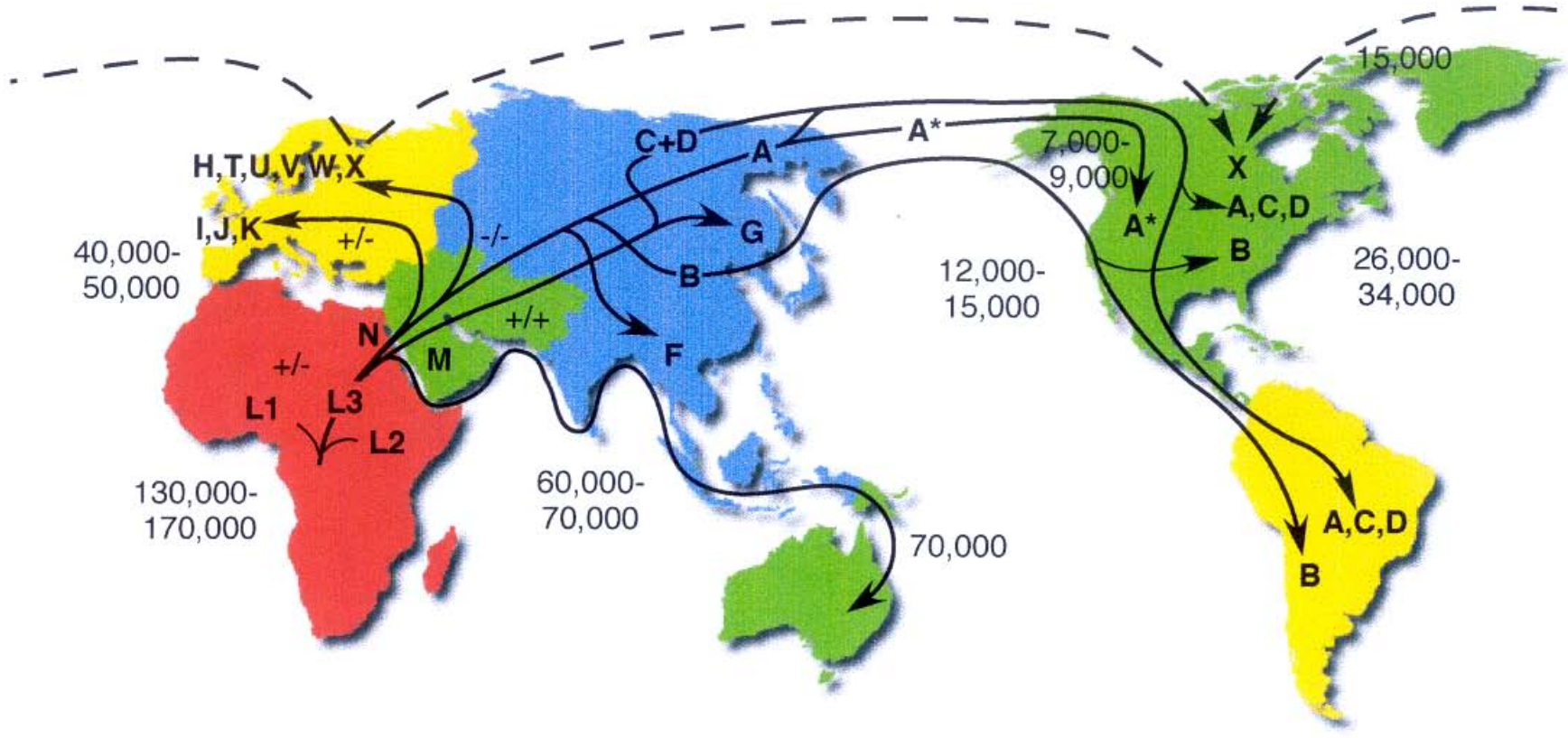
In 1997 Matthias Krings, Anne Stone, Ralf Schmitz, Heike Krainitzki, Mark Stoneking, and Svante Pääbo published the sequence of 379 base pairs of mtDNA extracted from a fossilized Neanderthal arm bone.[2] This particular fossil, discovered in 1856 near Dusseldorf, Germany, has been the subject of many intensive studies. After lengthy negotiations, the fossil's custodians granted Krings and co-workers permission to remove a 3.5-g piece of bone from the right humerus. Small fragments from this piece were pulverized, and the DNA remnants within them were carefully extracted. Because of the fossil's age (between 30,000 and 100,000 years), most of the DNA was expected to be degraded. However, because mtDNA is much more abundant than any particular sequence of nuclear DNA, Krings and co-workers hoped that some of it had survived. Their first step was to use a technique called the polymerase chain reaction (PCR, see Chapter 20) to amplify small segments of surviving mtDNA molecules. PCR allows a researcher to generate millions of identical DNA molecules from just a few molecules by *in vitro* replication with a bacterial DNA polymerase. The sequence of the amplified DNA can then be determined biochemically.

In carefully controlled experiments, Krings and coworkers succeeded in amplifying mtDNA remnants extracted from the fossil. Biochemical analysis of this ampli-

DNAs with shared polymorphisms are most closely related

Human mtDNA Migrations

http://www.mitomap.org/mitomap/WorldMigrations.pdf



+/-, +/+, or -/- = Dde I 10394 / Alu I 10397

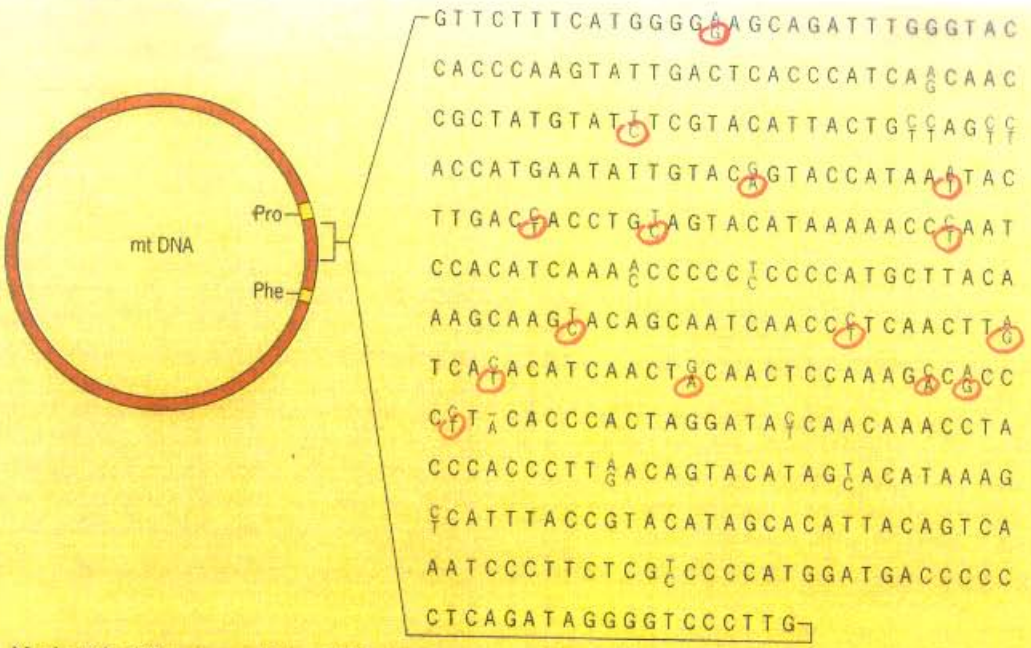
* = Rsa I 16329

Mutation rate = 2.2 - 2.9 % / MYR

Time estimates are YBP

13.a

NEANDERTHAL DNA SEQUENCES OBTAINED FROM BONES/FOSSILS USING PCR (ANCIENT DNA) INDICATE A SEPARATE LINE OF EVOLUTION



TOP Nt all HUMAN Nt DNA

Bottom Neanderthal Fossils

Figure 1. Nucleotide differences within a 379-bp noncoding region of the mtDNA of a Neanderthal fossil and that of a modern human being. The sequenced region lies between the genes for the phenylalanine (Phe) and proline (Pro) tRNAs. For each nucleotide difference (highlighted), the upper nucleotide is found in modern human mtDNA and the lower one is found in the Neanderthal mtDNA.

fied material showed that Neanderthal mtDNA differs from modern human mtDNA in 28 of the 379 nucleotides that were analyzed (Figure 1). The mtDNA isolated from different modern humans typically shows only 8 nucleotide substitutions in this region. Thus, Neanderthal mtDNA is significantly unlike that of modern humans. Computer analysis of the DNA sequences suggested that the human and Neanderthal mtDNA lineages began to evolve separately between 550,000 and 690,000 years ago, and that modern human mtDNAs originated between 120,000 and 150,000 years ago, apparently in Africa. Thus, Neanderthals were almost certainly not ancestral to modern humans. Rather, they evolved separately and, in the end, became extinct.

In the discussion section of their paper, Krings and coauthors concluded that "The Neanderthal mtDNA sequence thus supports a scenario in which modern humans arose recently in Africa as a distinct species and replaced Neanderthals with little or no interbreeding." They also added a caveat: "It must be emphasized that the above conclusions are based on a single individual sequence; the retrieval and analysis of mtDNA sequences from additional Neanderthal specimens is obviously desirable."[3] Of course, obtaining mtDNA sequences from other Neanderthals will entail the destruction of rare fossil material. Thus, the decision to collect such data should not be taken lightly. The benefit of collecting data from several individuals may not outweigh the cost of sacrificing so many valuable fossils. However, obtaining the sequence from at least one more Neanderthal does seem worthwhile, since this sequence could reinforce or invalidate the inferences that have to be made from the single sequence now available. We will have to wait and see if another Neanderthal fossil suitable for DNA analysis can be found. If it can, then the issue will be whether or not to allow part of that fossil to be destroyed to obtain a few molecules of mtDNA.

[1]Wilson, A. C., and R. L. Cann. 1992. The recent African genesis of humans. *Sci. Amer.*, 266(4):68–73.

[2]Krings, M., A. Stone, R. W. Schmitz, H. Krainitzki, M. Stoneking, and S. Pääbo. 1997. Neandertal DNA sequences and the origin of modern humans. *Cell* 90:19–30.

[3]ibid., p. 27.

Ancient DNA

USING MITOCHONDRIAL DNA IN FORENSICS – CZAR'S CHILDREN

MATERNAL / FAMILY RELATIONS

TECHNICAL SIDELIGHT

DNA Tests and the Mystery of the Duchess Anastasia

According to historical records, the Russian royal family—Tsar Nicholas II, Tsarina Alexandra, and their five children: Alexis, Olga, Tatiana, Marie, and Anastasia (Figure 1)—were executed on July 16, 1918, by a revolutionary Bolshevik firing squad and then were buried in a single grave in the Ural Mountains. However, in 1920, an unknown woman, "Fraulein Unbekannt," who was pulled from a canal in Berlin in a state of hypothermia, claimed that she was the Duchess Anastasia. Although she did not speak Russian, Fraulein Unbekannt, or Anna Anderson Manahan, as she was subsequently known, was amazingly well informed about details of life in the imperial Russian court. Her claim to be Anastasia was vigorously rejected by the surviving relatives of the Russian royal family. The Grand Duke of Hesse even hired a private detective to investigate Anna's heritage. The detective concluded that Anna was really Franzisca Schanzkowska, but the dispute continued. Although little is known about Franzisca, she was born in the northern part of Germany, lived in Berlin during World War I, and was severely injured by an explosion while working in a munitions factory. She was subsequently admitted to two mental hospitals for treatment. She disappeared in 1920, about the same time that Anna Anderson Manahan was rescued from the Berlin canal and claimed to be Anastasia.

When Princess Irene of Prussia, Anastasia's aunt, was persuaded to meet with the woman who claimed to be her niece, Anna ran and hid in her room. Anna's bizarre behavior made her claim to be Anastasia difficult to evaluate, and the controversy over the identity of Anna Anderson Manahan continued for over 70 years. Was Anna really Anastasia? Her supporters were steadfast in their belief that she was indeed the Duchess. Disbelievers were equally adamant that she was not Anastasia.

In 1979, a Russian geologist discovered a shallow grave believed to contain the remains of the royal family. Because of the political climate in the Soviet Union at the time, the geologist reburied the bodies. Twelve years later, when the political climate was more favorable, the bodies were exhumed, and their authenticity was established by comparing DNA from the skeletons with DNA from surviving relatives. However, the controversy about the identity of Anna was rekindled by the absence of two bodies, those of Anastasia and her brother Alexis. Had they somehow escaped execution? Although there is still no definitive answer to this question, the results of recent DNA tests indicate that Anna Anderson Manahan was not the Duchess Anastasia.

Anna Anderson Manahan died in 1984 at the age of 83. However, during surgery performed in 1979 at the Martha Jefferson Hospital in Charlottesville, Virginia, intestinal tissues were removed, fixed in formaldehyde, and preserved

Figure 1 The children of Tsar Nicholas II: (left to right) Marie, Tatiana, Anastasia, Olga, and Alexis.

in paraffin blocks. In addition, a few of Anna's hair follicles were preserved. DNA tests—VNTR (variable number tandem repeat) prints and nucleotide sequences of specific noncoding regions of mitochondrial DNA—were performed on these preserved tissues and on relatives of Franzisca Schanzkowska and of the royal family. These tests were performed independently in three different laboratories: (1) the Armed Forces DNA Identification Laboratory in the United States, (2) the Forensic Science Service in the United Kingdom, and (3) the Department of Anthropology at Pennsylvania State University. The results obtained by the three laboratories all indicate that Anna Anderson Manahan was not Anastasia. Indeed, the results strongly suggest that Anna was Franzisca Schanzkowska.

Of five different VNTRs examined, four were inconsistent with the possibility that Anna was the daughter of Tsar Nicholas II and Tsarina Alexandra. DNA sequence comparisons also argued that Anna was not related to the royal family. Instead, the nucleotide sequence data indicated that Anna was Ms. Schanzkowska. At the six variable positions shown below, Anna's mitochondrial DNA contained the same nucleotides as the DNA from Carl Maucher, Franzisca Schanzkowska's grand nephew, and differed from those in the DNA of the Duke of Edinburgh, the grand nephew of Tsarina Alexandra.

	Variable Nucleotides in Mitochondrial DNA					
Position:	**1**	**2**	**3**	**4**	**5**	**6**
Anna Anderson Manahan	C	C	T	T	C	T
Carl Maucher (Grand nephew of Franzisca)	C	C	T	T	C	T
Duke of Edinburgh (Grand nephew of Alexandra)	T	T	C	C	T	C

NEED Mt DNA + SEQUENCE OF REGION!

"Real" Princess Recognized

THE HUMAN GENOME SEQUENCE

articles

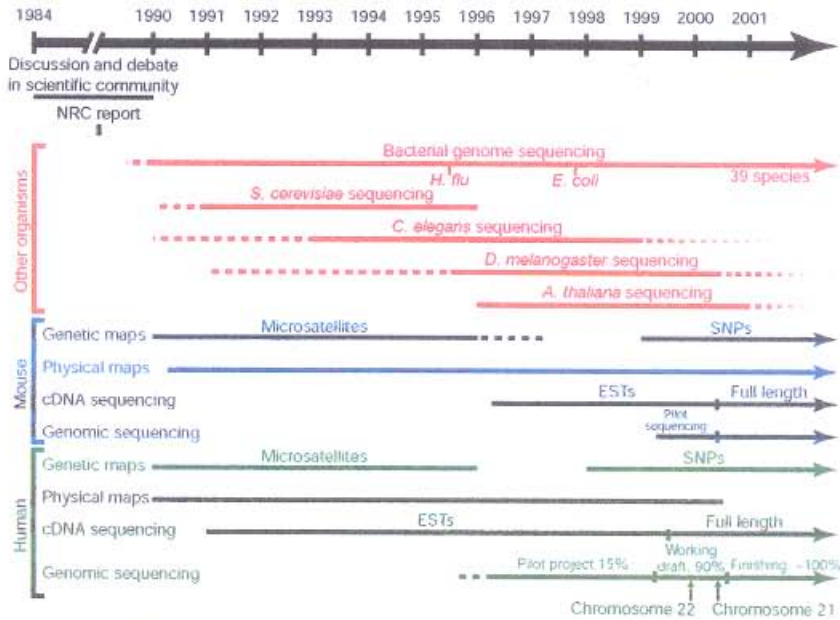
Initial sequencing and analysis of the human genome

International Human Genome Sequencing Consortium*

* *A partial list of authors appears on the opposite page. Affiliations are listed at the end of the paper.*

The human genome holds an extraordinary trove of information about human development, physiology, medicine and evolution. Here we report the results of an international collaboration to produce and make freely available a draft sequence of the human genome. We also present an initial analysis of the data, describing some of the insights that can be gleaned from the sequence.

NATURE | VOL 409 | 15 FEBRUARY 2001 | www.nature.com



DONE!

Figure 1 Timeline of large-scale genomic analyses. Shown are selected components of work on several non-vertebrate model organisms (red), the mouse (blue) and the human (green) from 1990; earlier projects are described in the text. SNPs, single nucleotide polymorphisms; ESTs, expressed sequence tags.

16

WITHOUT AUTOMATION THE HUMAN GENOME COULD NOT HAVE BEEN SEQUENCED

PRODUCTION LINE

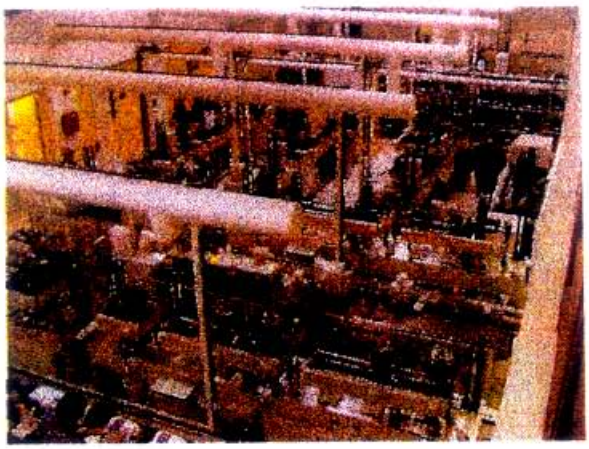


Figure 3 The automated production line for sample preparation at the Whitehead Institute, Center for Genome Research. The system consists of custom-designed factory-style conveyor belt robots that perform all functions from purifying DNA from bacterial cultures through setting up and purifying sequencing reactions.

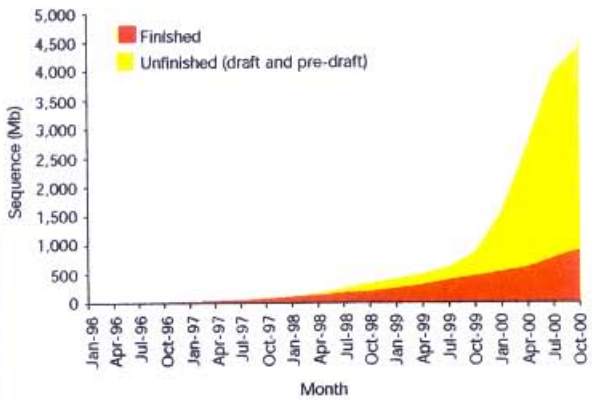


Figure 4 Total amount of human sequence in the High Throughput Genome Sequence (HTGS) division of GenBank. The total is the sum of finished sequence (red) and unfinished (draft plus predraft) sequence (yellow).

THE HUMAN GENOME SEQUENCE IS THE RESULT OF AN INTERNATIONAL COLLABORATION

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12

AND COMPLETED IN ONLY Nine Months!

IT WAS A RACE!

Celera Effort

5 people's DNA combined + Sequenced

3♀ 2♂

2 Caucasian
1 African American
1 Chinese
1 Hispanic

A 2.91-billion base pair (bp) consensus sequence of the euchromatic portion of the human genome was generated by the whole-genome shotgun sequencing method. The 14.8-billion bp DNA sequence was generated over 9 months from 27,271,853 high-quality sequence reads (5.11-fold coverage of the genome) from both ends of plasmid clones made from the DNA of five individuals. Two assembly strategies—a whole-genome assembly and a regional chromosome assembly—were used, each combining sequence data from Celera and the publicly funded genome effort. The public data were shredded into 550-bp segments to create a 2.9-fold coverage of those genome regions that had been sequenced, without including biases inherent in the cloning and assembly procedure used by the publicly funded group. This brought the effective coverage in the assemblies to eightfold, reducing the number and size of gaps in the final assembly over what would be obtained with 5.11-fold coverage. The two assembly strategies yielded very similar results that largely agree with independent mapping data. The assemblies effectively cover the euchromatic regions of the human chromosomes. More than 90% of the genome is in scaffold assemblies of 100,000 bp or more, and 25% of the genome is in scaffolds of 10 million bp or larger. Analysis of the genome sequence revealed 26,588 protein-encoding transcripts for which there was strong corroborating evidence and an additional ~12,000 computationally derived genes with mouse matches or other weak supporting evidence. Although gene-dense clusters are obvious, almost half the genes are dispersed in low G+C sequence separated by large tracts of apparently noncoding sequence. Only 1.1% of the genome is spanned by exons, whereas 24% is in introns, with 75% of the genome being intergenic DNA. Duplications of segmental blocks, ranging in size up to chromosomal lengths, are abundant throughout the genome and reveal a complex evolutionary history. Comparative genomic analysis indicates vertebrate expansions of genes associated with neuronal function, with tissue-specific developmental regulation, and with the hemostasis and immune systems. DNA sequence comparisons between the consensus sequence and publicly funded genome data provided locations of 2.1 million single-nucleotide polymorphisms (SNPs). A random pair of human haploid genomes differed at a rate of 1 bp per 1250 on average, but there was marked heterogeneity in the level of polymorphism across the genome. Less than 1% of all SNPs resulted in variation in proteins, but the task of determining which SNPs have functional consequences remains an open challenge.

1% Exons
24% Introns
75% Intergenic
100% DNA

2005
5.8×10^6 SNPs
or
1/~600 bp
on average between
2 people

1 SNP/1250 bp on average between two people

BUT The International Public Sequence is More Complete than the Private one That contains Gaps

READ –

"The GENOME WAR"

by James Shreeve

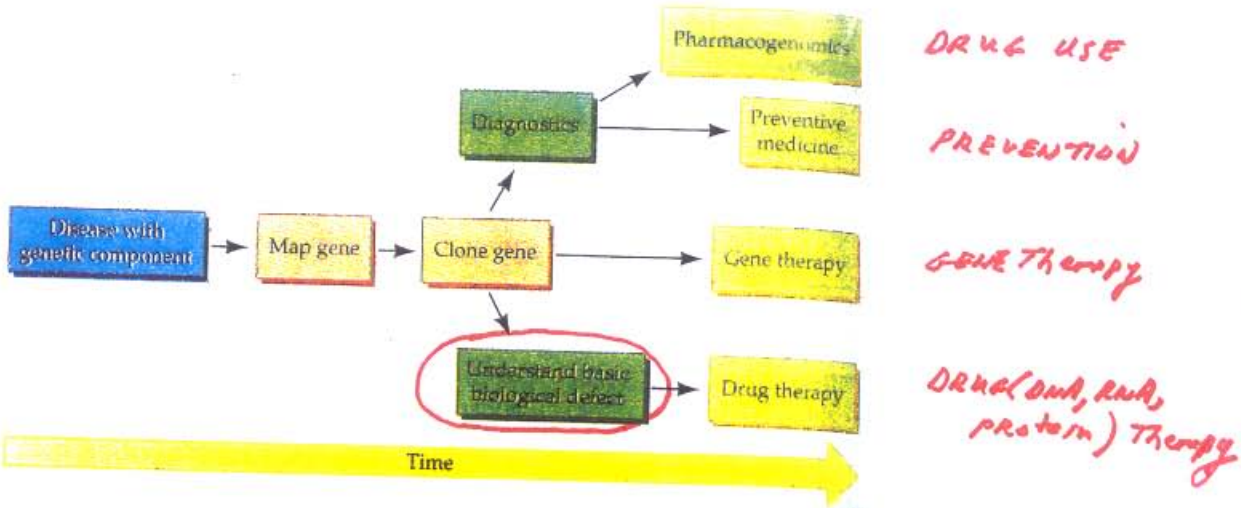
ISBN 0375406298

JANUARY, 2004

Private vs. Public Genome Projects!

KNOWLEDGE OF THE HUMAN GENOME WILL REVOLUTIONIZE MEDICINE

BASIC KNOWLEDGE DRIVES APPLICATIONS!



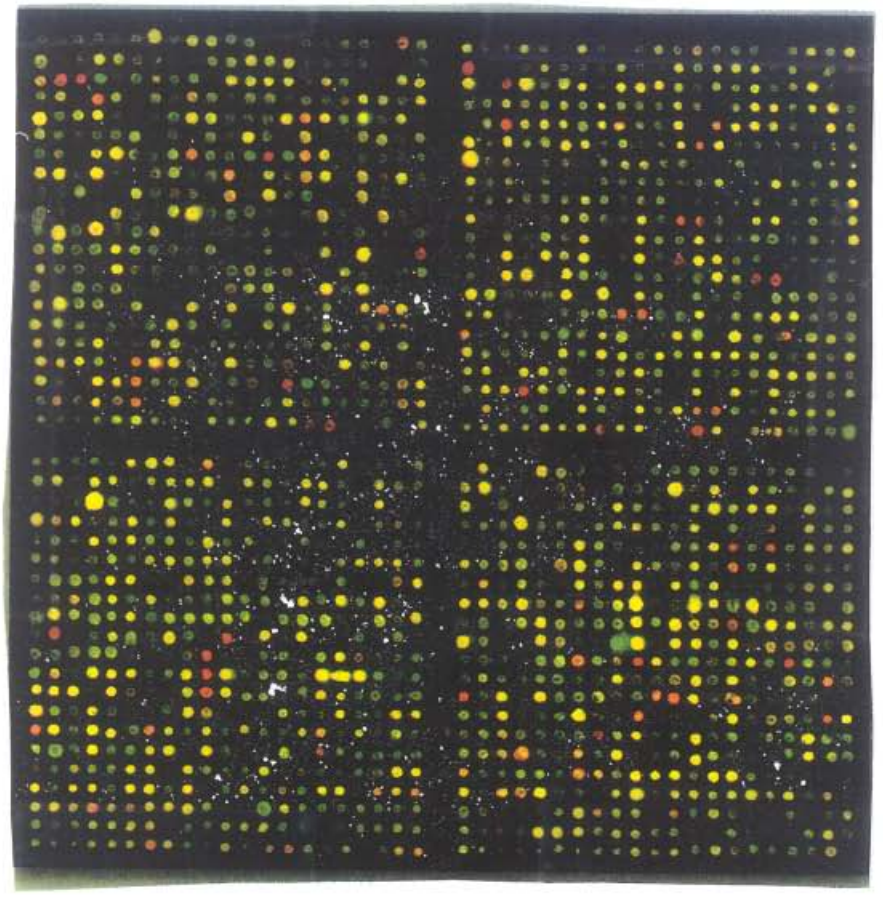
18.22 Is This the Future of Medicine?
The elucidation of the human genome sequence may result in an approach to medicine that is oriented to the genetic and functional individuality of each patient.

PERSONAL, PROACTIVE, PREVENTIVE

ASSAY FOR PERSON-SPECIFIC GENES!

SEQUENCE ANY GENOME FOR $1000 IN 10 years!

INDIVIDUAL GENE PROFILES WILL BECOME POSSIBLE



Heart Disease ? Predisposition !

Drug utility ? Metabolism !

CANCER ? Early detection / Therapy !

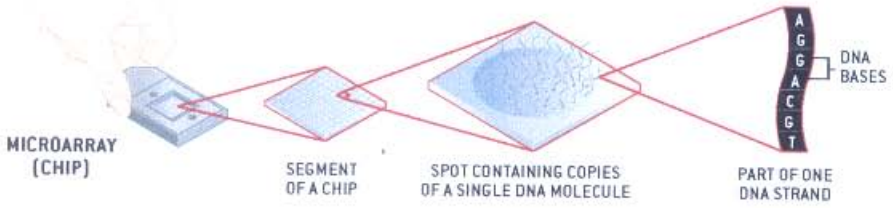
etc

Whole-GENOME CHIPS/MICROARRAYS

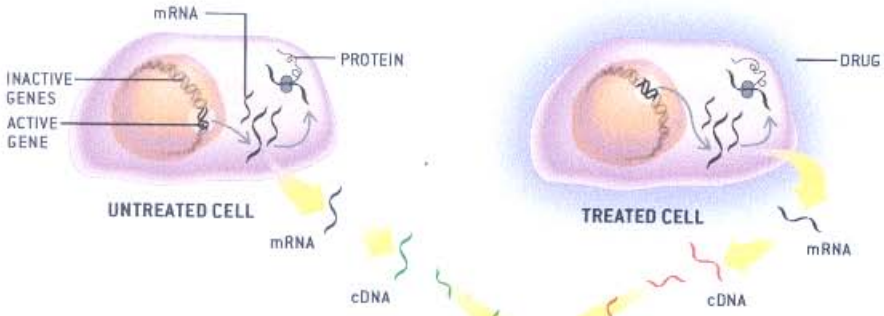
Whole-GENOME EXPRESSION

HOW ARRAYS WORK

TO DETERMINE QUICKLY whether a potential new drug is likely to harm the liver, a researcher could follow the steps below, asking this question: Does the drug cause genes (the blueprints for proteins) in liver cells to alter their activity in ways that are known to cause or reflect liver damage? A "yes" answer would be a sign of trouble.



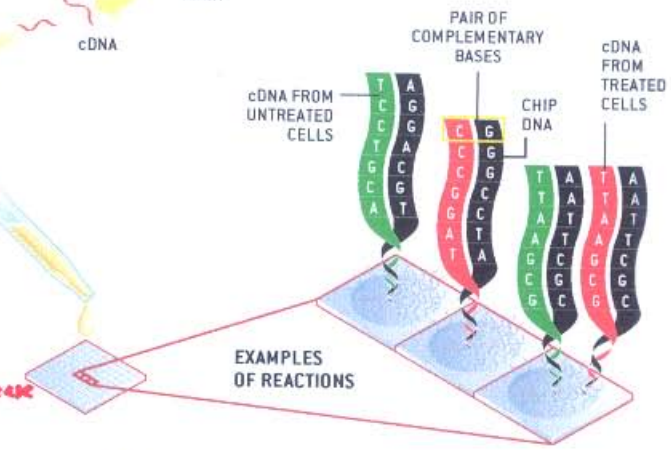
1 Construct or buy a microarray, or chip, containing single-stranded DNA representing thousands of different genes, each assigned to a specified spot on the one-by-three-inch or smaller device. Have every spot include thousands to millions of copies of a DNA strand.



2 Obtain two samples of liver cells; apply the drug to one sample. Then, from each sample, collect molecules of messenger RNA (mRNA)—the mobile copies of genes and the templates for protein synthesis in cells.

3 Transcribe the mRNA into more stable complementary DNA (cDNA) and add fluorescent labels—green to cDNAs derived from untreated cells, red to those from treated cells.

4 Apply the labeled cDNAs to the chip. Binding occurs when cDNA from a sample finds its complementary sequence of bases on the chip (*detail at right*). Such binding means that the gene represented by the chip DNA was active, or expressed, in the sample.

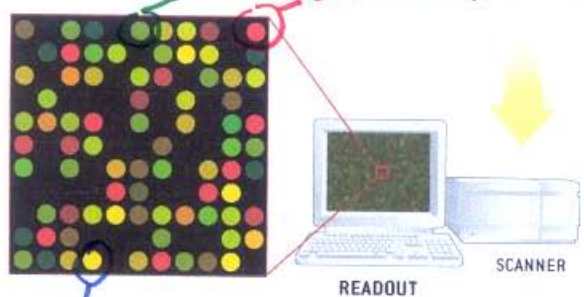


Key

- GENE THAT STRONGLY INCREASED ACTIVITY IN TREATED CELLS
- GENE THAT STRONGLY DECREASED ACTIVITY IN TREATED CELLS
- GENE THAT WAS EQUALLY ACTIVE IN TREATED AND UNTREATED CELLS
- GENE THAT WAS INACTIVE IN BOTH GROUPS

decrease

Increase

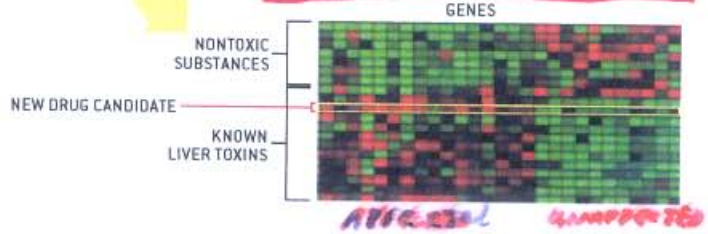


same

5 Put the chip in a scanner. Have a computer calculate the ratio of red to green at each spot (to quantify any changes in gene activity induced by the drug) and generate a color-coded readout.

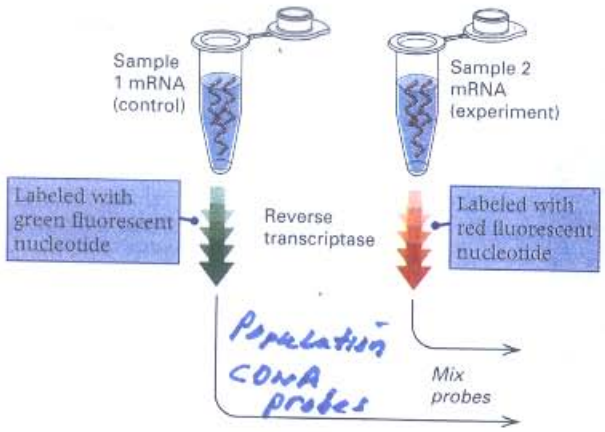
AFFECTED UNAFFECTED

HYPOTHETICAL PROFILES OF GENE ACTIVITY IN CELLS TREATED WITH VARIOUS COMPOUNDS



6 Determine whether any genes responded strongly to the drug in ways known to promote or reflect liver damage. Or compare the overall expression pattern produced by strong responders with the patterns produced when those genes react to known liver toxins (*right*). Close similarity would indicate that the new candidate was probably toxic as well. In the diagram, each box represents a single gene's response to a compound.

USING WHOLE GENOME CHIPS TO STUDY ACTIVITY OF ALL GENES IN GENOME TOGETHER



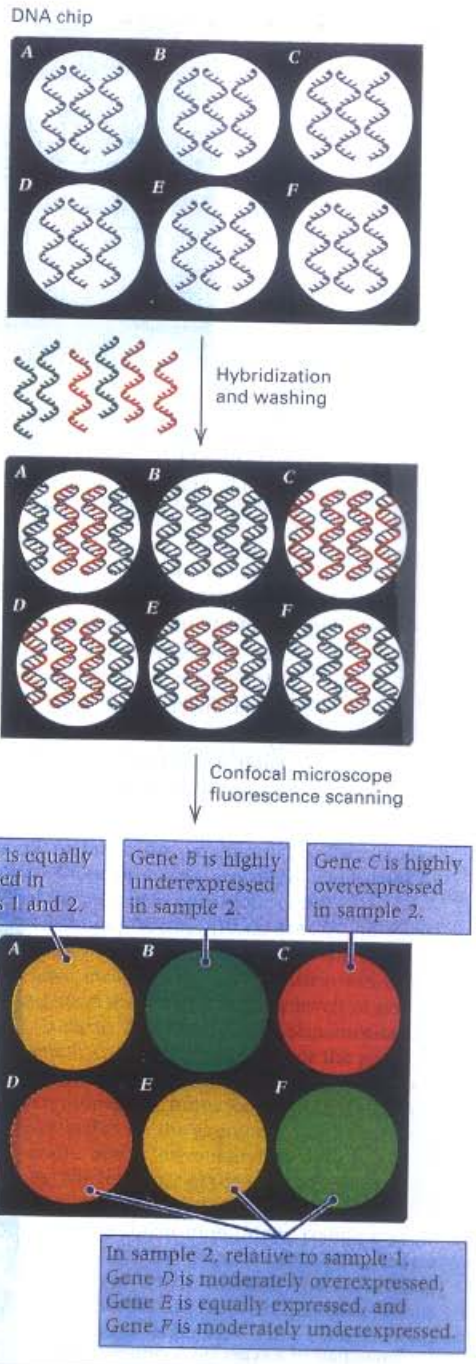


Figure 13.30 Principle of operation of one type of DNA chip. At the top are dried microdrops, each of which contains immobilized DNA strands from a different gene ($A-F$). These are hybridized with a mixture of fluorescence-labeled DNA samples obtained by reverse transcription of cellular mRNA. Competitive hybridization of red (experimental) and green (control) label is proportional to the relative abundance of each mRNA species in the samples. The relative levels of red and green fluorescence of each spot are assayed by microscopic scanning and displayed as a single color. Red or orange indicates overexpression in the experimental sample, green or yellow-green underexpression in the experimental sample, and yellow equal expression.

Genes A, B, C, D, E, & F in mRNAs 1 & 2

High in mRNA$_1$

High in mRNA$_2$

Yellow = in mRNAs 1 & 2

Fluorescent nucleotides

The HUMAN GENOME IS LARGE — BUT NOT THE LARGEST GENOME!

Scale = 60 nts / 10cm

What is the HUMAN GENOME LENGTH? 3×10^9 bp in meters?

1 Meter per haploid genome!

Figure 1.4 The immense length of the human genome.

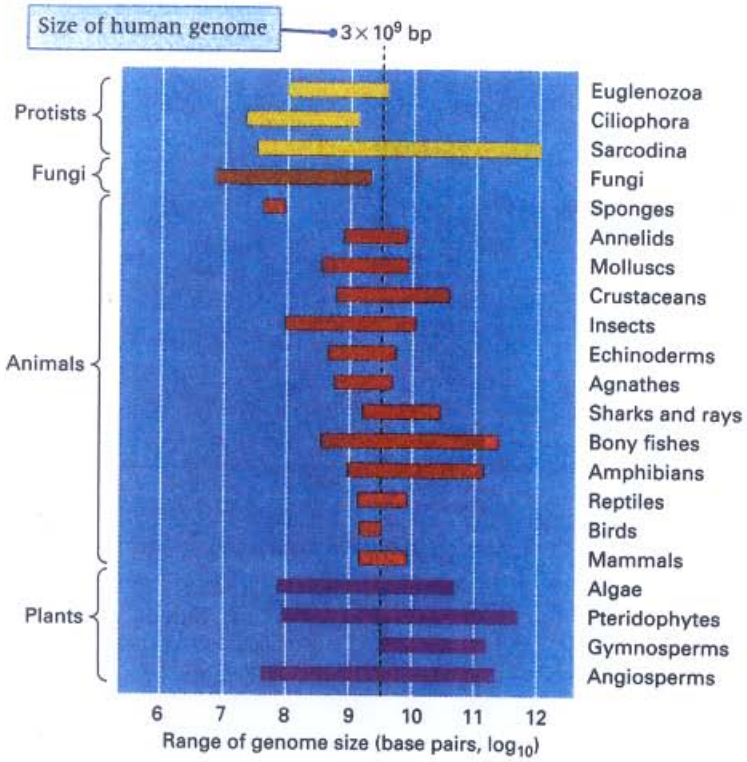


Figure 8.1 Genome size ranges over several orders of magnitude in some groups of organisms, and genome size is not correlated with developmental, metabolic, or behavioral complexity.

1. *kilobase (kb)*	10^3 nucleotide pairs (double-stranded) or 10^3 nucleotides (single-stranded)
2. *megabase (Mb)*	10^6 nucleotide pairs (double-stranded) or 10^6 nucleotides (single-stranded)
3. *gigabase (Gb)*	10^9 nucleotide pairs (double-stranded) or 10^9 nucleotides (single-stranded)

"TOP DOWN" & "BOTTOM UP" APPROACHES TO Whole-GENOME DNA Sequencing

TOP DOWN

BOTTOM UP

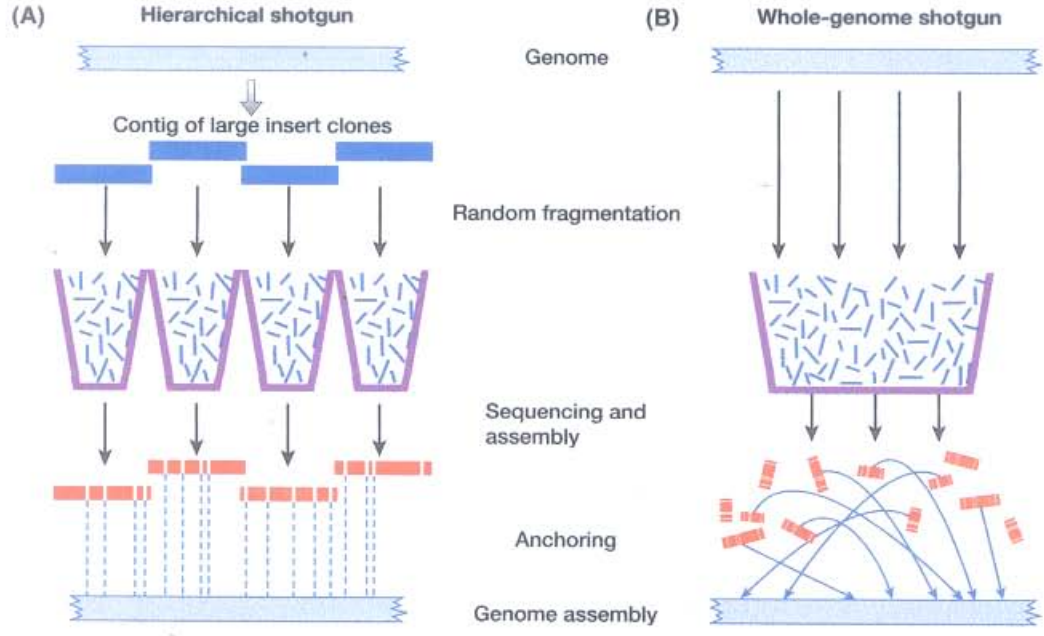


Figure 8.3: Different shotgun sequencing strategies for sequencing the human genome.

(A) ***Hierarchical shotgun sequencing***. Human genomic DNA is fragmented by partial restriction digestion and the resulting large restriction fragments are cloned into BAC vectors to generate a BAC library. BAC clones are organized into large contigs by typing all clones with STS markers to identify clones with overlapping inserts. The inserts of selected BAC clones are shotgun cloned and sequenced. The sequenced fragments of a BAC are then assembled to give the BAC sequence and the full BAC sequences are integrated to remove overlaps.
(B) ***Whole genome shotgun sequencing***. Here isolated genomic DNA is submitted directly to shotgun cloning and sequencing, and the sequenced pieces are assembled into large contigs spanning megabases. Adapted from Waterston *et al.* (2002) *Proc. Natl Acad. Sci. USA* **99**, p. 3713, with permission from the National Academy of Sciences, USA.

TOP DOWN

Physical Map of Genome
Sequence
Annotate
Takes Longer / More Accurate

BOTTOM UP

Random Sequence
Assemble into Contigs
Annotate / Super Computers
Faster / Gaps

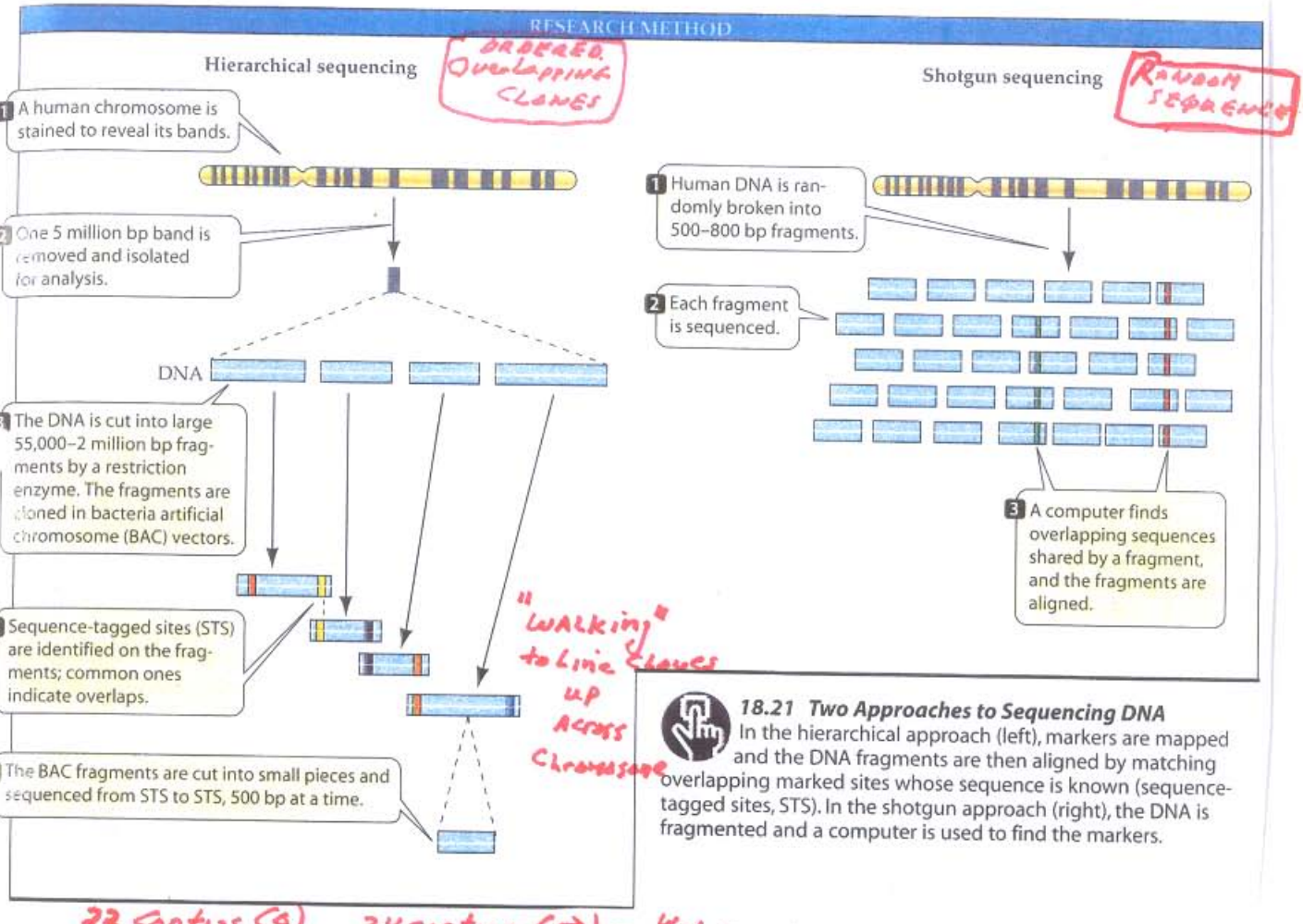
HOW WAS THE HUMAN GENOME SEQUENCED?

TOP DOWN

Physical Map → Sequence

BOTTOM UP

Sequence → Assemble



18.21 Two Approaches to Sequencing DNA In the hierarchical approach (left), markers are mapped and the DNA fragments are then aligned by matching overlapping marked sites whose sequence is known (sequence-tagged sites, STS). In the shotgun approach (right), the DNA is fragmented and a computer is used to find the markers.

23 contigs (♀) 24 contigs (♂) — Y chromosome

PUBLIC EFFORT

CHROMOSOME WALKS → SEQUENCE

BEST
MOST COMPLETE
ENTIRE
SLOW

PRIVATE EFFORT

SHOTGUN

FAST
MANY GAPS
SKELETON

NEEDS PUBLIC DATA TO ASSEMBLE → CHROMOS

TOP DOWN SEQUENCING OF THE HUMAN GENOME

www.ncbi.nih.gov/entrez

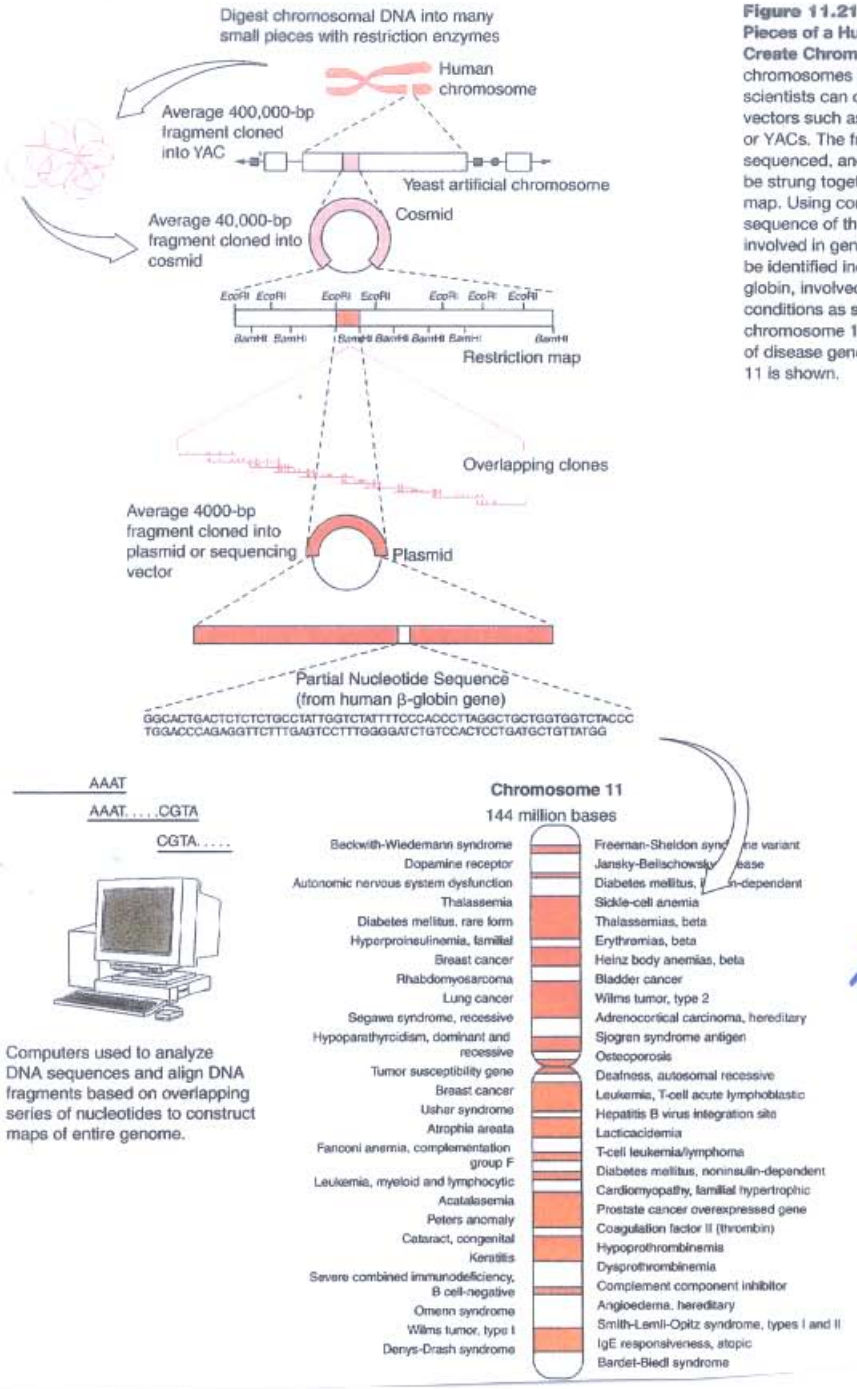


Figure 11.21 Cloning and Sequencing Pieces of a Human Chromosome to Create Chromosome Maps By cutting chromosomes into small pieces, genome scientists can clone these pieces into vectors such as plasmids, cosmids, BACs, or YACs. The fragments can then be sequenced, and the overlapping pieces can be strung together to make a continuous map. Using computers to analyze the sequence of these fragments, genes involved in genetic disease conditions can be identified including genes, such as globin, involved in genetic disease conditions as shown in this map of chromosome 11. Note: Only a partial map of disease genes located on chromosome 11 is shown.

NOTE -

BANDS specific FOR specific DNA Sequence

Correlate with disease genes / find them!

OMIM™ - ONLine Mendelian Inheritance in Man

The PUBLIC TOP DOWN APPROACH

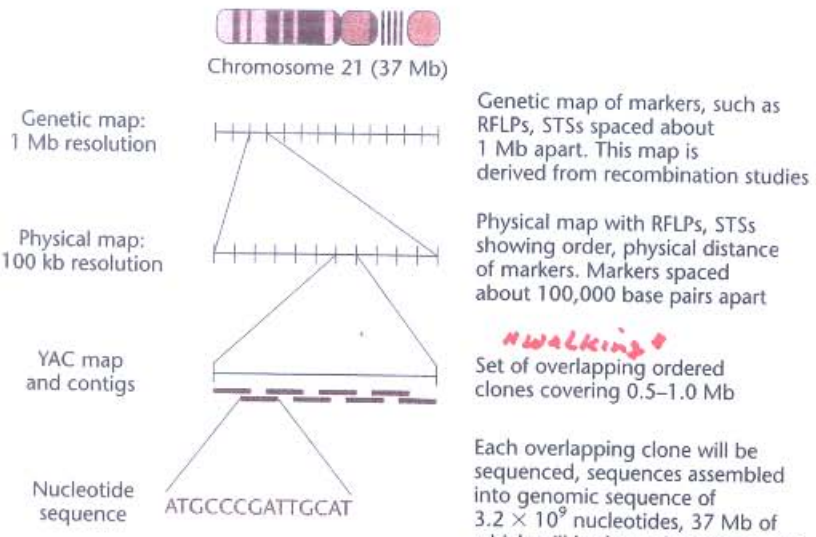


FIGURE 21.17 An overview of the strategy used in the Human Genome Project. The first goal, achieved in 1995, was to have a genetic map of each chromosome, with markers spaced at distances of about 1 Mb (1 million base pairs of DNA). This work was accomplished by finding markers such as RFLPs and STSs and assigning them to chromosomes. Once assigned to chromosomes, the markers' inheritance was observed in heterozygous families to establish the order and distance between them (a genetic map). In the second stage, the goal was to prepare a physical map of each chromosome (our example uses chromosome 21, the smallest chromosome) containing the location of markers spaced about 100,000 base pairs apart. This goal has now been achieved. The third stage involves the construction of a set of overlapping clones, in yeast artificial chromosomes (YACs) or other vectors that cover the length of the chromosome. The last stage will be the sequencing of the entire genome. Sequencing on selected parts of the genome has started.

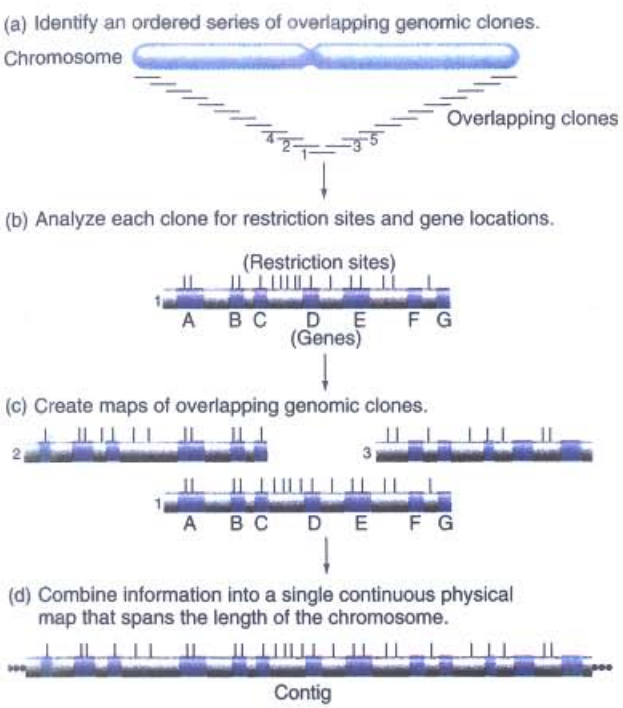


Figure 10.5 Building a whole-chromosome physical map. (a) To produce a whole-chromosome physical map, you first order a set of overlapping genomic clones that extend from one end of the chromosome to the other. Subsequent figures describe various methods of obtaining this ordered set of clones. (b) You next map the restriction sites of each clone in the set through restriction analysis, and analyze individual restriction fragments in other ways, such as Northern blot analysis, to identify transcription units. (c) Computers overlay the different types of maps for each clone onto the overlapping clones to obtain a continuous map. (d) The result is a single continuous map extending the length of the chromosome.

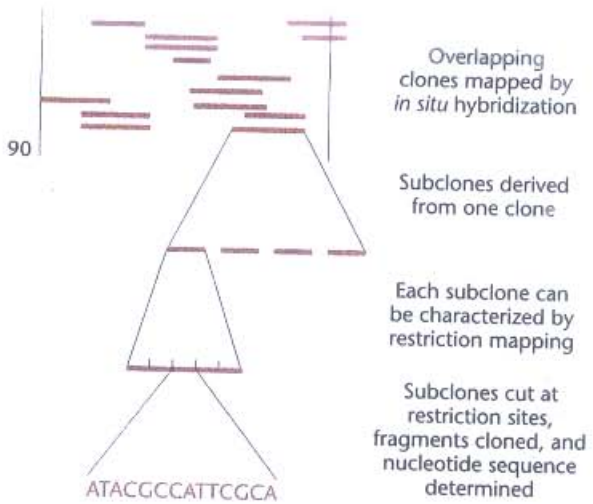


FIGURE 20.2 The top-down approach for the *Drosophila* genome project. A genome library is constructed with very large fragments (~200 kb) in a special vector. The physical location of each is mapped to the polytene chromosomes. Each clone is then broken down into subclones, which are characterized by restriction mapping for DNA sequence analysis.

Note: Smaller & Smaller Libraries of overlapping clones know where clone is in genome :: know sequence of that region!

FINDING GENES WITHIN AN "OCEAN" OF DNA Sequences Using Bioinformatics

Figure 8.6: Gene finding by computer-based analysis of genomic sequences.

This example shows ***NIX analysis*** (see text) of a PAC sequence from a region of chromosome 12q24.1 encompassing the Darier's disease locus. The nucleotide size of the region is illustrated by the bar at the bottom. Analyses include the use of programs to scan for gene-associated motifs such as promoter sequences (green inverted triangles at top), and polyadenylation sites (ochre-colored inverted triangles) and various exon prediction programs (GRAIL, GENSCAN etc.). Significant homologies to other sequences at the nucleotide level and at the protein level are indicated by the boxes for the various BLAST programs. Data provided by Dr. Victor Ruiz-Perez and Simon Carter, University of Newcastle upon Tyne, UK.

Using Signature Sequences & Genetic Code
Yo! It's in the DNA!

MAPPING DISEASE GENES TO SPECIFIC REGIONS OF THE HUMAN GENOME

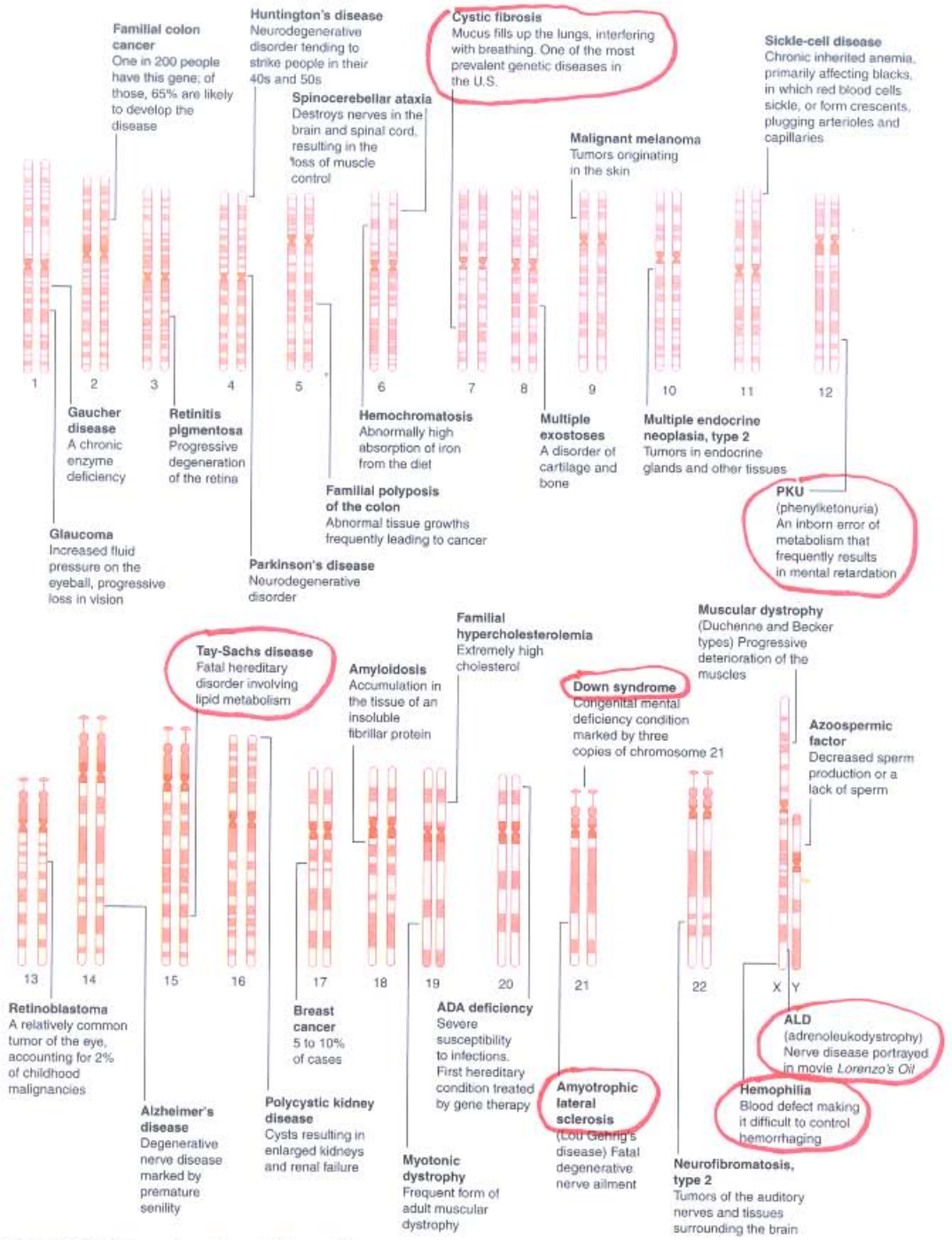


Figure 11.22 Disease Gene Maps of Human Chromosomes Maps show one or two genes on each human chromosome that are involved in a genetic condition. Many more genes than are shown in this figure are located on each chromosome. Note: Chromosomes are not drawn to scale.

OMIM™

SEQUENCING HUMAN CHROMOSOMES

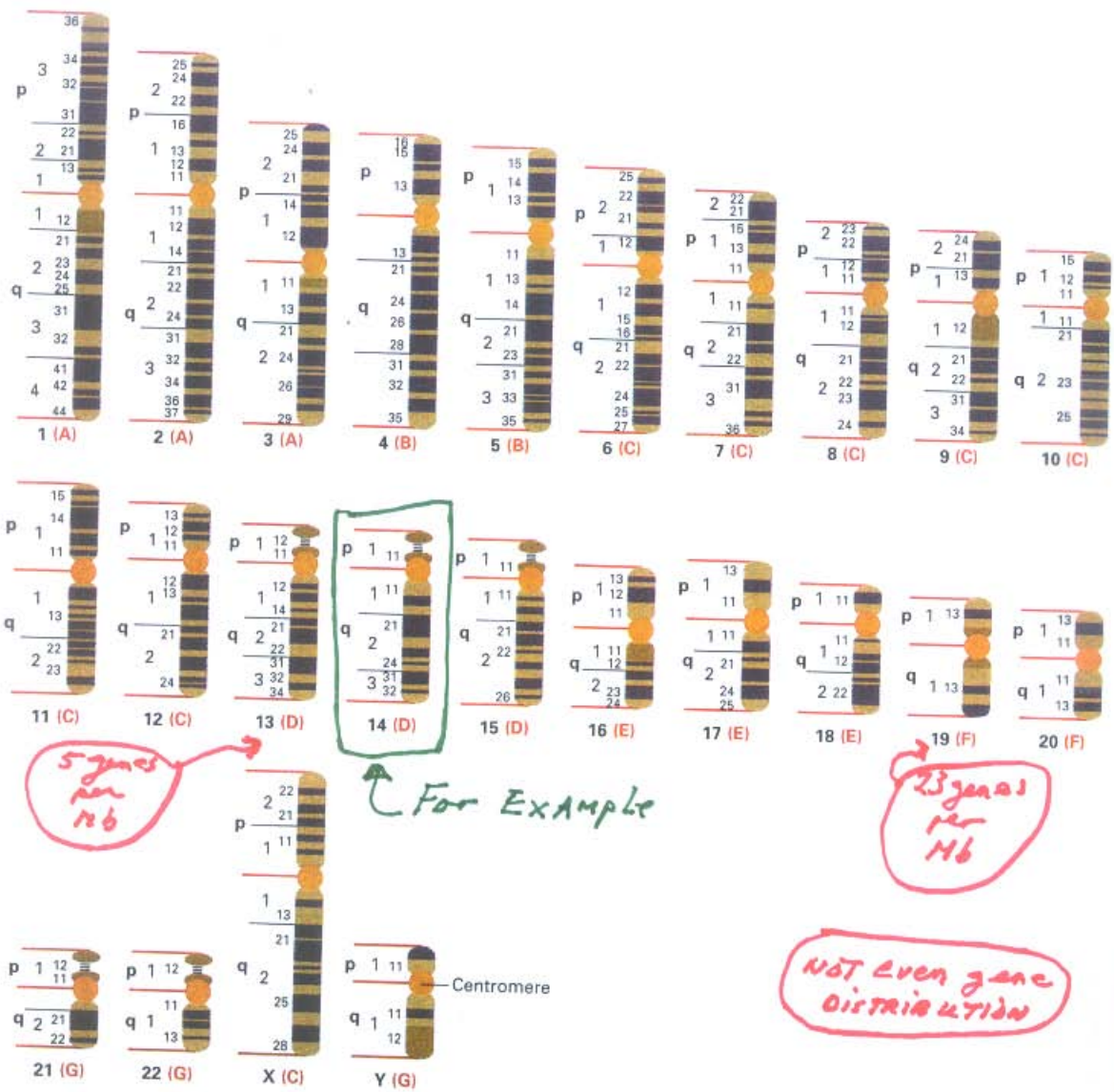


Figure 9.3 Designations of the bands and interbands in the human karyotype. Beneath each chromosome is the lettered group (A–G) to which it belongs.

SYMBOLS USED TO "MAP" HUMAN KARYOTYPE/CHROMOSOMES

Table 9.1 Conventional karyotype symbols used in human genetics

A–G	Chromosome groups
1–22	Autosome designations
X, Y	Sex-chromosome designations
p	Short arm of chromosome
q	Long arm of chromosome
ter	Terminal portion: pter refers to terminal portion of short arm, qter to terminal portion of long arm
+	Preceding a chromosome designation, indicates that the chromosome or arm is extra; following a designation, indicates that the chromosome or arm is larger than normal
−	Preceding a chromosome designation, indicates that the chromosome or arm is missing; following a designation, indicates that the chromosome or arm is smaller than normal
mos	Mosaic
/	Separates karyotypes of clones in mosaics—e.g., 47, XXX/45,X
dup	Duplication
dir dup	Direct duplication
inv dup	Inverted duplication
del	Deletion
inv	Inversion
t	translocation
rcp	Reciprocal translocation
rob	Robertsonian translocation
r	Ring chromosome
i	Isochromosome (two identical arms attached to a single centromere, like an attached-X chromosome in *Drosophila*)

CHROMOSOME TO DNA SEQUENCE TO GENES!

SEQUENCING CHROMOSOME 14 TOP DOWN

REGIONS/BANDS →

Cytogenetic map

BANDS

14p11.1-14q11.1 | 14q11.2 | 14q12 | 14q13.1 | 14q13.2 | 14q13.3

Genetic markers

CLONES

Contig tiling path

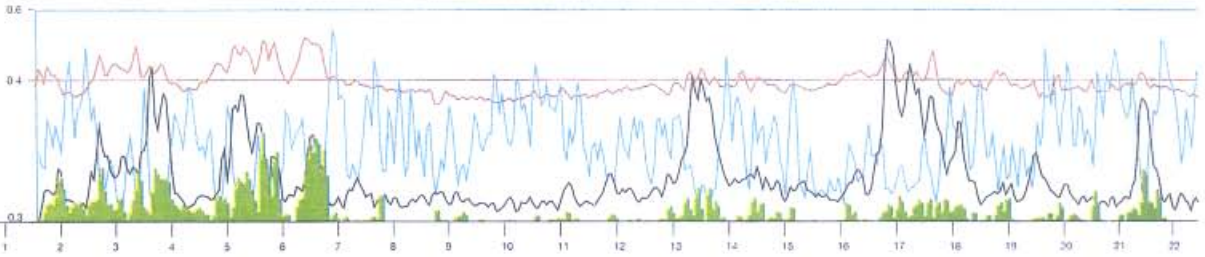
WALKING

Experimental sequence validation

Intrachromosomal duplication

Interchromosomal duplication

(G+C) content



Scale (Mb)

GENES → WATSON

Genes (Forward)

BIOINFORMATICS GENBANK

Known genes

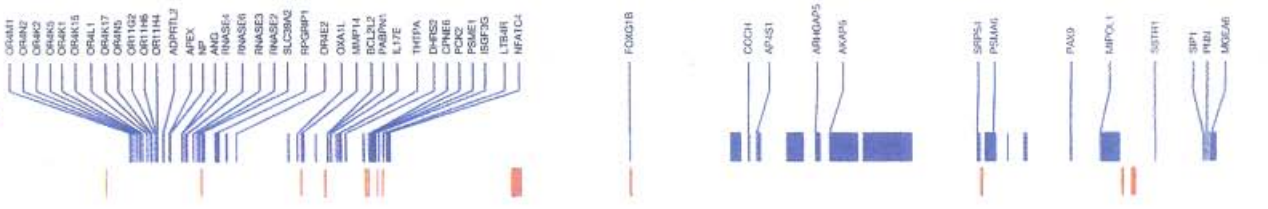
Novel genes

Novel transcripts

Putative genes

Predicted genes

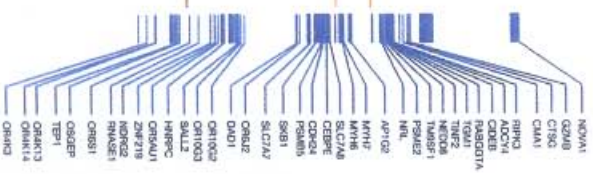
Pseudogenes



CpG islands

Genes (Reverse)

GENES ← CRICK



TCR locus

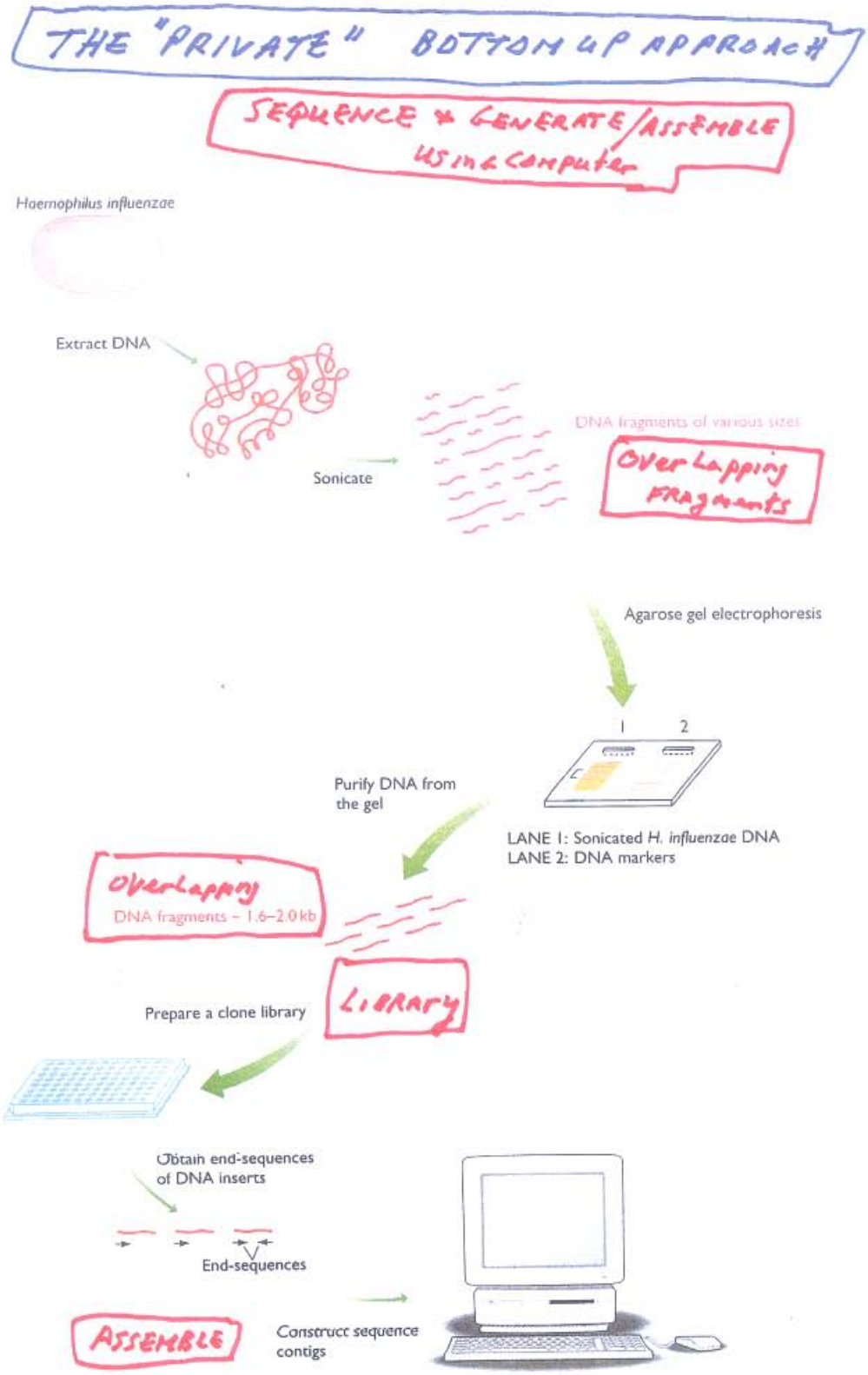


Figure 4.10 The way in which the shotgun approach was used to obtain the DNA sequence of the *Haemophilus influenzae* genome.

H. influenzae DNA was sonicated and fragments with sizes between 1.6 and 2.0 kb purified from an agarose gel and ligated into a plasmid vector to produce a clone library. End-sequences were obtained from clones taken from this library, and a computer used to identify overlaps between sequences. This resulted in 140 sequence contigs, which were assembled into the complete genome sequence as shown in *Figure 4.11*. For further details, see Fleischmann *et al.*, 1995.

APPROXIMATELY 39,000 GENES HAVE BEEN IDENTIFIED IN THE HUMAN GENOME

Table 11. Genome overview.

Size of the genome (including gaps)	2.91 Gbp
Size of the genome (excluding gaps)	2.66 Gbp
Longest contig	1.99 Mbp
Longest scaffold	14.4 Mbp
Percent of A+T in the genome	54
Percent of G+C in the genome	38
Percent of undetermined bases in the genome	9
Most GC-rich 50 kb	Chr. 2 (66%)
Least GC-rich 50 kb	Chr. X (25%)
Percent of genome classified as repeats	35
Number of annotated genes	26,383
Percent of annotated genes with unknown function	42
Number of genes (hypothetical and annotated)	39,114
Percent of hypothetical and annotated genes with unknown function	59
Gene with the most exons	Titin (234 exons)
Average gene size	27 kbp
Most gene-rich chromosome	Chr. 19 (23 genes/Mb)
Least gene-rich chromosomes	Chr. 13 (5 genes/Mb), Chr. Y (5 genes/Mb)
Total size of gene deserts (>500 kb with no annotated genes)	605 Mbp
Percent of base pairs spanned by genes	25.5 to 37.8*
Percent of base pairs spanned by exons	1.1 to 1.4*
Percent of base pairs spanned by introns	24.4 to 36.4*
Percent of base pairs in intergenic DNA	74.5 to 63.6*
Chromosome with highest proportion of DNA in annotated exons	Chr. 19 (9.33)
Chromosome with lowest proportion of DNA in annotated exons	Chr. Y (0.36)
Longest intergenic region (between annotated + hypothetical genes)	Chr. 13 (3,038,416 bp)
Rate of SNP variation	1/1250 bp

*In these ranges, the percentages correspond to the annotated gene set (26, 383 genes) and the hypothetical + annotated gene set (39,114 genes), respectively.

3x10⁹ bp (2.91 x 10⁹) SAME AS MOUSE & CHIMP!

42% of Genes/Unknown Function!

CHROMOSOMES VARY in Gene Density

1/1250 bp in 2005 — Polymorphisms!

PROTOONCOGENE 3% ~1000 GENES

6% TRANSCRIPTION FACTORS = SWITCH REGULATORS ~2000 GENES

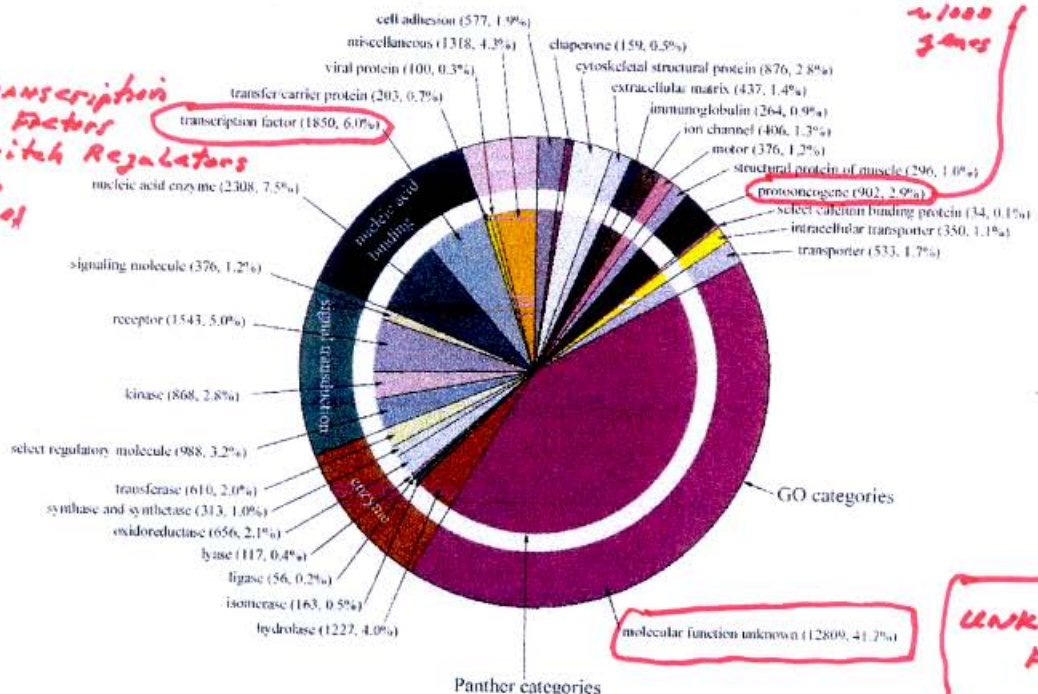


Fig. 15. Distribution of the molecular functions of 26,383 human genes. Each slice lists the numbers and percentages (in parentheses) of human gene functions assigned to a given category of molecular function. The outer circle shows the assignment to molecular function categories in the Gene Ontology (GO) (*179*), and the inner circle shows the assignment to Celera's Panther molecular function categories (*116*).

UNKNOWN GENE FUNCTIONS 42%! ~13,000 genes!

The HUMAN GENOME CONTAINS The SAME NUMBER OF GENES AS A WEED!!

Table 23 Properties of genome and proteome in essentially completed eukaryotic proteomes

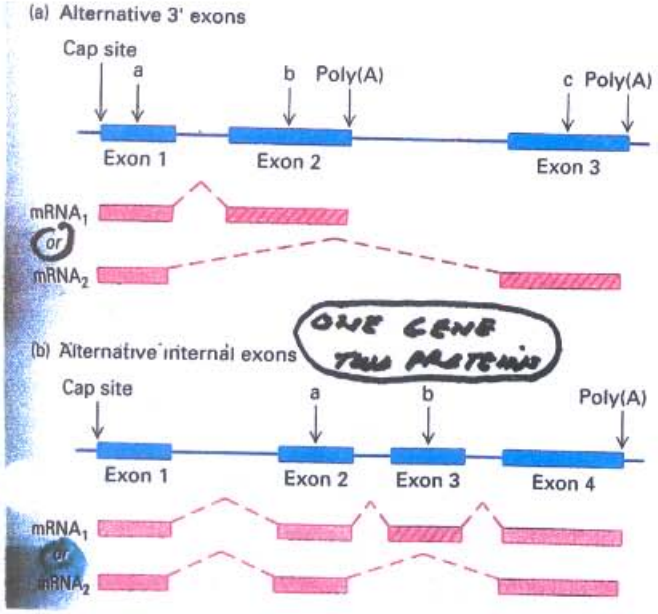
	Human	Fly	Worm	Yeast	Mustard weed
Number of identified genes	~32,000*	13,338	18,266	6,144	25,706
% with InterPro matches	51	56	50	50	52
Number of annotated domain families	1,262	1,035	1,014	851	1,010
Number of InterPro entries per gene	0.53	0.84	0.63	0.6	0.62
Number of distinct domain architectures	1,695	1,036	1,018	310	-
Percentage of 1-1-1-1	1.40	4.20	3.10	9.20	-
% Signal sequences	20	20	24	11	-
% Transmembrane proteins	20	25	28	15	-
% Repeat-containing	10	11	9	5	-
% Coiled-coil	11	13	10	9	-

The numbers of distinct architectures were calculated using SMART[339] and the percentages of repeat-containing proteins were estimated using Prospero[150] and a *P*-value threshold of 10^{-5}. The protein sets used in the analysis were taken from http://www.ebi.ac.uk/proteome/ for yeast, worm and fly. The proteins from mustard weed were taken from the TAIR website (http:// www.arabidopsis.org/) on 5 September 2000. The protein set was searched against the InterPro database (http://www.ebi.ac.uk/interpro/) using the InterProscan software. Comparison of protein sequences with the InterPro database allows prediction of protein families, domain and repeat families and sequence motifs. The searches used Pfam release 5.2[307], Prints release 26.1[305], Prosite release 16[307] and Prosite preliminary profiles. InterPro analysis results are available as Supplementary Information. The fraction of 1-1-1-1 is the percentage of the genome that falls into orthologous groups composed of only one member each in human, fly, worm and yeast.

*The gene number for the human is still uncertain (see text). Table is based on 31,778 known genes and gene predictions.

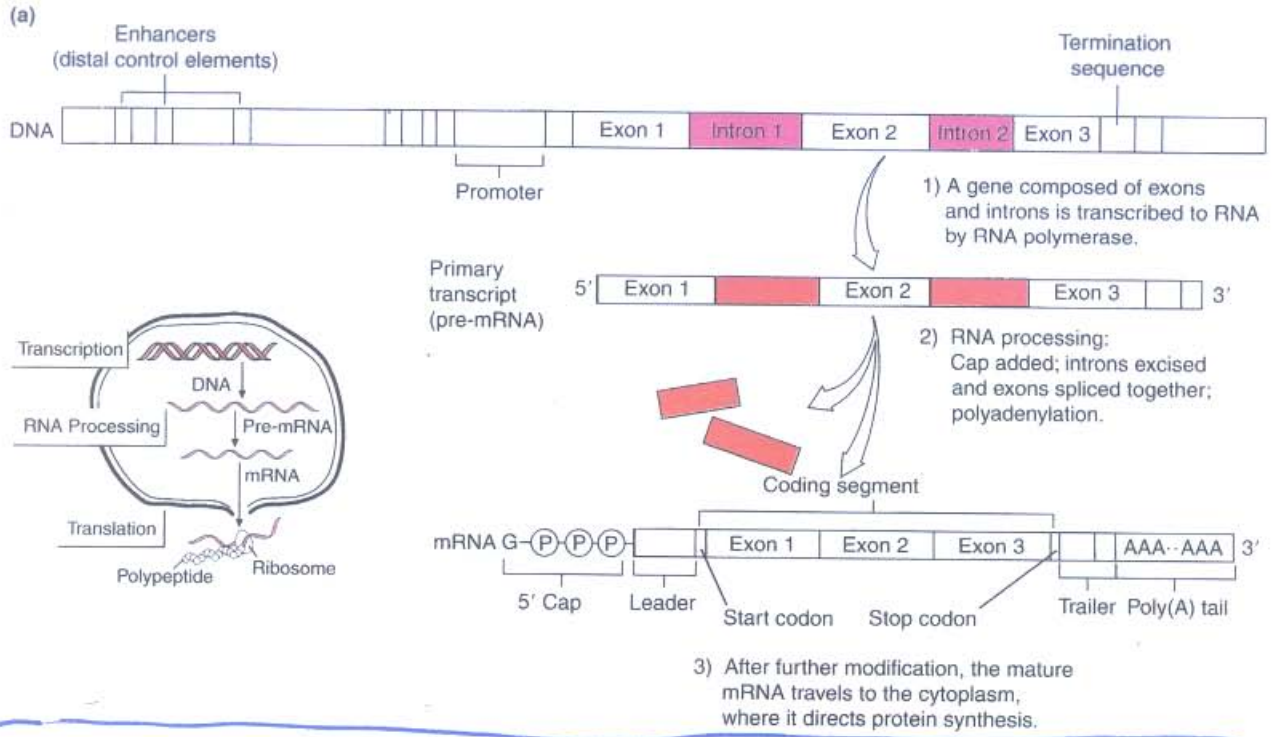
BUT REMEMBER – PROTEINS PRODUCE the Phenotype

∴ The Potential to MAKE MANY MORE THOUSANDS of Proteins EXISTS ..... Alternate Splicing!



▲ FIGURE 9-2 Two examples of complex eukaryotic transcription units and the effect of mutations on expression of the encoded proteins. The RNA transcribed from a complex transcription unit (blue) can be processed in alternative ways to yield two or more functional monocistronic mRNAs. Dashed lines indicate spliced-out introns. (a) A complex transcription unit whose primary transcript has two poly(A) sites produces two mRNAs with alternative 3′ exons. (b) A complex transcription unit whose primary transcript undergoes exon skipping during processing produces alternative mRNAs with the same 5′ and 3′ exons. In this example, some cell types would express the mRNA including exon 3, whereas in other cell types, exon 2 is spliced to exon 4, producing an mRNA lacking exon 3 and the protein sequence it encodes. In (a) and (b), mutations (designated *a*) within exons shared by the alternative mRNAs (solid red) affect the proteins encoded by both alternatively processed mRNAs. In contrast, mutations (designated *b* and *c*) within exons unique to one of the alternatively processed mRNAs (red with diagonal lines) affect only the protein encoded by that mRNA.

The Human Genome Encodes More Proteins than Genes because of Differential Splicing of Primary Transcripts



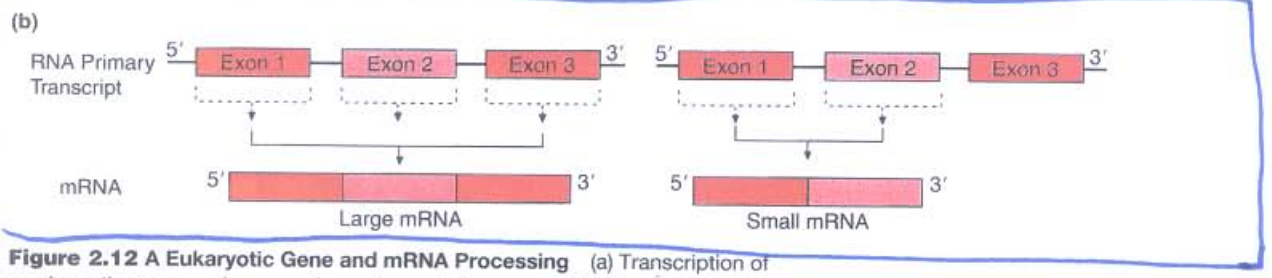


Figure 2.12 A Eukaryotic Gene and mRNA Processing (a) Transcription of a eukaryotic gene produces a primary transcript or pre-mRNA. The primary transcript undergoes processing through RNA splicing, the addition of a 5′ cap, and polyadenylation. After processing, the final, mature mRNA is ready for export to the cytoplasm where it will be translated into a protein. (b) Alternative splicing can produce different mRNAs and protein products from the same gene. Notice that the larger mRNA on the left contains three exons spliced together but that the shorter mRNA on the right contains only two exons spliced together.

one 1° Transcript several mRNAs!

35,000 human genes can potentially encode 100,000 – 200,000 "different" proteins

Approximately Half of the Genes in the Human Genome have Unknown Functions

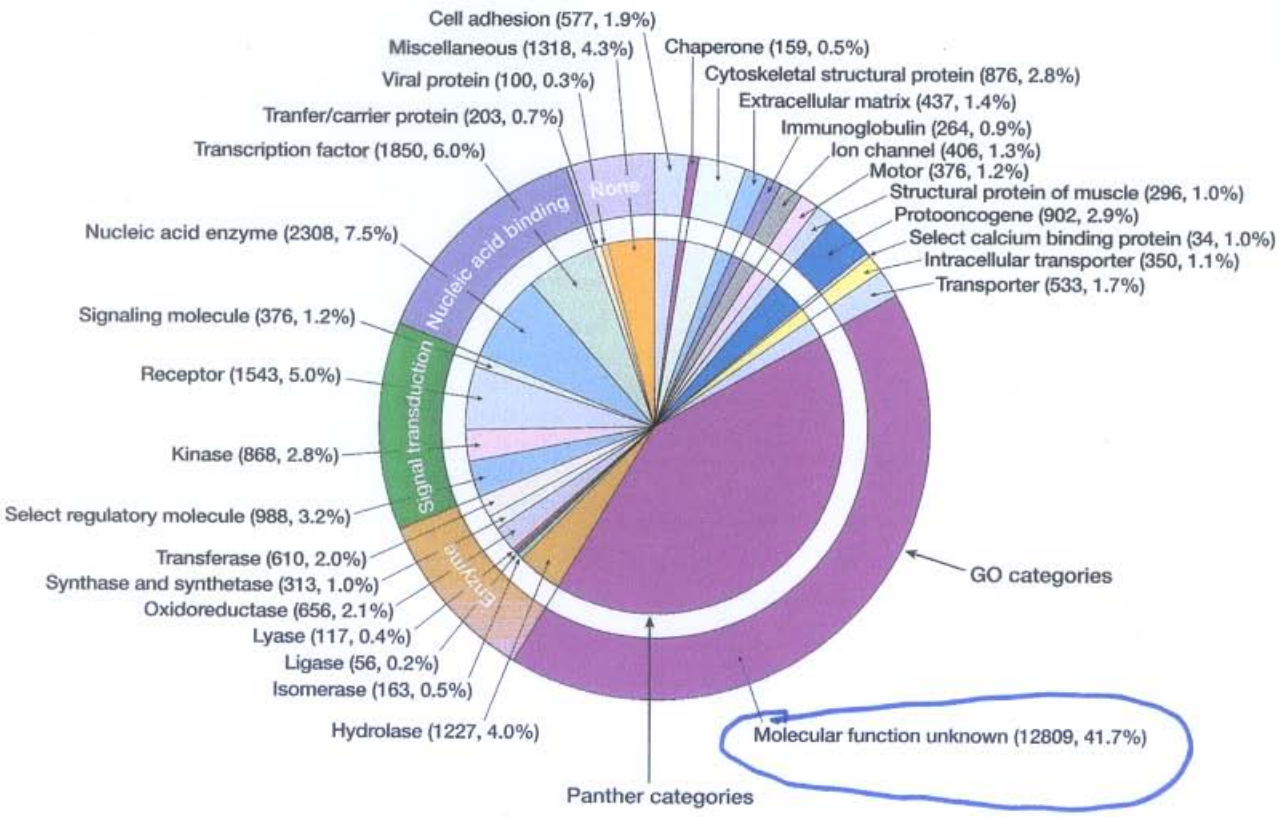


Figure 12.20: A preliminary functional classification of human polypeptide-encoding genes.

Known or predicted functions for 26 383 human polypeptide-encoding genes. Classification is according to the GO molecular function categories as shown in the outer circle (Gene Ontology classification – see Section 8.3.6) or to Celera's Panther molecular function categories (inner circle). Reproduced from Venter *et al.* (2001) *Science* **291**, 1304–1351, with permission from the American Association for the Advancement of Science.

Lots of work yet to do!

Use Mouse as Model to Identify Unknown Genes functions!!

HUMAN GENES CAN BE VERY LARGE!

WITH MANY INTRONS!

articles

Table 21 Characteristics of human genes

	Median	Mean	Sample (size)
Internal exon	122 bp	145 bp	RefSeq alignments to draft genome sequence, with confirmed intron boundaries (43,317 exons)
Exon number	7	8.8	RefSeq alignments to finished sequence (3,501 genes)
Introns	1,023 bp	3,365 bp	RefSeq alignments to finished sequence (27,238 introns)
3′ UTR	400 bp	770 bp	Confirmed by mRNA or EST on chromosome 22 (689)
5′ UTR	240 bp	300 bp	Confirmed by mRNA or EST on chromosome 22 (463)
Coding sequence (CDS)	1,100 bp	1,340 bp	Selected RefSeq entries (1,804)
	367 aa	447 aa	
Genomic extent	14 kb	27 kb	Selected RefSeq entries (1,804)

Median and mean values for a number of properties of human protein-coding genes. The 1,804 selected RefSeq entries were those that could be unambiguously aligned to finished sequence over their entire length.

Titin Gene = 363 Exons Encodes Muscle Protein 38,000 aa's i. mRNA = 114kb! 114,000 b.!

Table 7.7: Average sizes of exons and introns in human genes

Gene product	Size of gene (kb)	Number of exons	Average size of exon (bp)	Average size of intron (bp)
$tRNA^{Tyr}$	0.1	2	50	20
Insulin	1.4	3	155	480
β-Globin	1.6 SMALL!	3	150	490
Class I HLA	3.5	8	187	260
Serum albumin	18	14	137	1100
Type VII collagen	31	118	77	190
Complement C3	41	29	122	900
Phenylalanine hydroxylase	90	26	96	3500
Factor VIII	186 0.2Mb	26	375	7100
CFTR (cystic fibrosis)	250	27	227	9100
Dystrophin	2400 2.4Mb	79	180	30 000

Note – Small exons Large in trons!

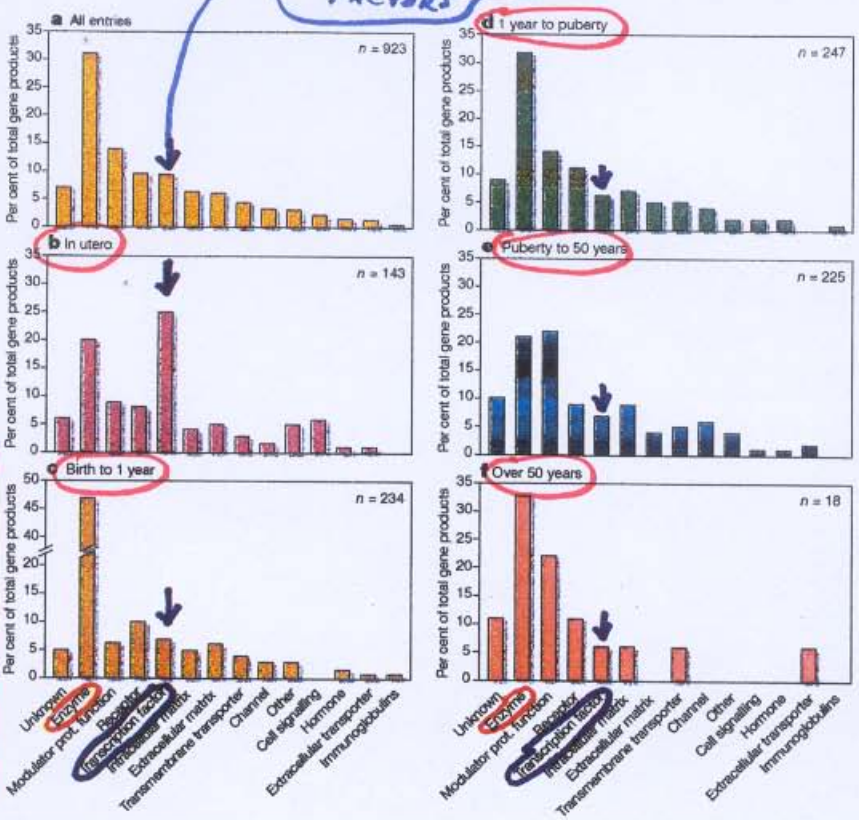
Note: tiny Exons in "sea" of huge introns!

MANY DISEASE GENES HAVE BEEN IDENTIFIED

AND what their Proteins Are!

TRANSCRIPTION FACTORS

onset of disease



note! Most Disease Genes Encode Enzymes & Affect Metabolism e.g. ADA gene

GARROD's INBORN ERRORS OF Metabolism

Figure 1 The functions of the protein products of disease genes. **a**, The entire disease gene set. **b–f**, Disease genes stratified according to the typical age of onset of the disease phenotype. The fraction of disease genes encoding transcription factors in the *in utero* onset disorders (25%) differs from the fraction encoding transcription factors for disorders with onset after birth (6%; $\chi^2 = 49.4$, $P < 0.001$). Similarly, the fraction of disease genes encoding enzymes causing a disorder with onset in the first year of life (47%) is different from the fraction encoding enzymes causing disorders with other ages of onset (25.8%; $\chi^2 = 35.8$, $P < 0.001$).

10,000 Disease Genes

Defects in Genes Encoding Regulatory Proteins cause disease

Defects in enzyme Genes Cause Defects

AND HOW THEY ARE INHERITED, WHEN DISEASE BEGINS, & HOW Life Expectancy Affected!

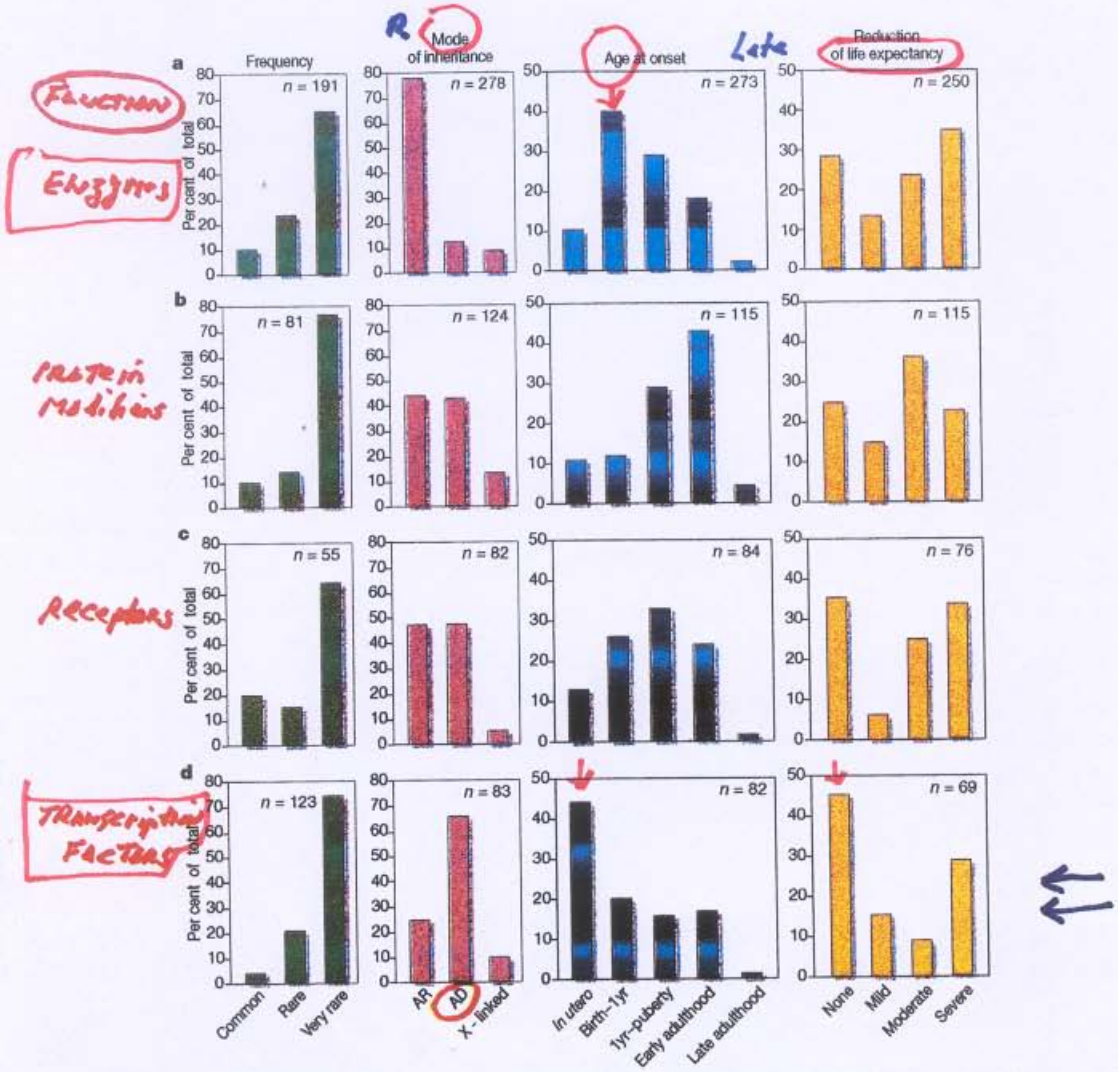


Figure 2 Characteristics of disease arranged by function of the protein encoded by the disease gene. **a**, Disease genes encoding enzymes; **b**, disease genes encoding modifiers of protein function; **c**, disease genes encoding receptors; **d**, disease genes encoding transcription factors. The columns of disease features are labelled at the top. AR, autosomal recessive; AD, autosomal dominant; early adulthood, puberty to <50 years; late adulthood, >50 years.

Clearly – These Genes CAN BE ASSAYED using Probes/Markers!

What Makes A MOUSE A MOUSE AND A PERSON A PERSON?!

75,000,000 years APART

TABLE 22.1 Comparison of Mice and Humans

Trait	Mice	Humans
Average weight	30 g	77,000 g (170 lb)
Average length	10 cm (without tail)	175 cm
Genome size	~3,000,000,000 bp	~3,000,000,000 bp
Haploid gene number	~100,000	~100,000
Number of chromosomes	19 autosomes + X and Y	22 autosomes + X and Y
Gestation period	3 weeks	38 weeks (8.9 months)
Age at puberty	5–6 weeks	624–728 weeks (12–14 years)
Estrus cycle	4 days	28 days
Life span	2 years	78 years

Mouse + Man diverged ~75,000,000 years ago!

LARGE BLOCKS OF MOUSE GENES ARE FOUND IN HUMAN CHROMOSOMES!

Note: Blocks of Mouse Genes in Human Chromosomes

HUMAN CHROMOSOMES

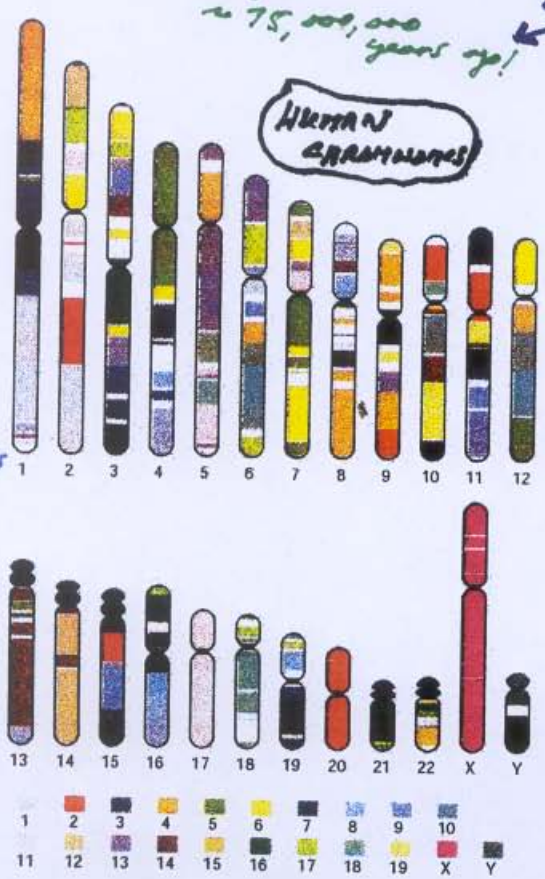


Figure 46 Conserved segments in the human and mouse genome. Human chromosomes, with segments containing at least two genes whose order is conserved in the mouse genome as colour blocks. Each colour corresponds to a particular mouse chromosome. Centromeres, subcentromeric heterochromatin of chromosomes 1, 9 and 16, and the repetitive short arms of 13, 14, 15, 21 and 22 are in black.

BLOCKS OF CONSERVED GENES

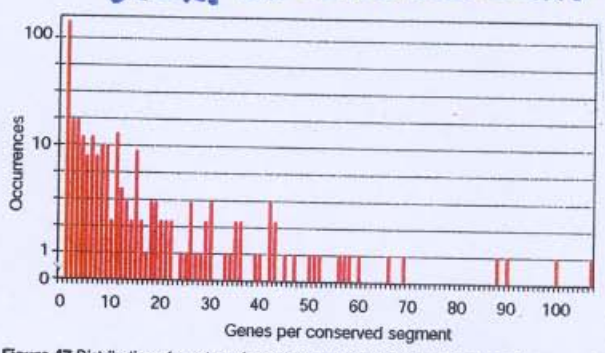


Figure 47 Distribution of number of genes per conserved segment between human and mouse genomes.

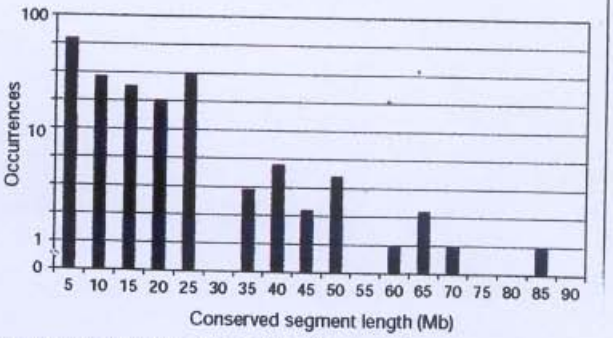


Figure 48 Distribution of lengths (in 5-Mb bins) of conserved segments between human and mouse genomes, omitting singletons.

99% of ALL HUMAN GENES ARE FOUND in the MOUSE GENOME!

Initial sequencing and comparative analysis of the mouse genome

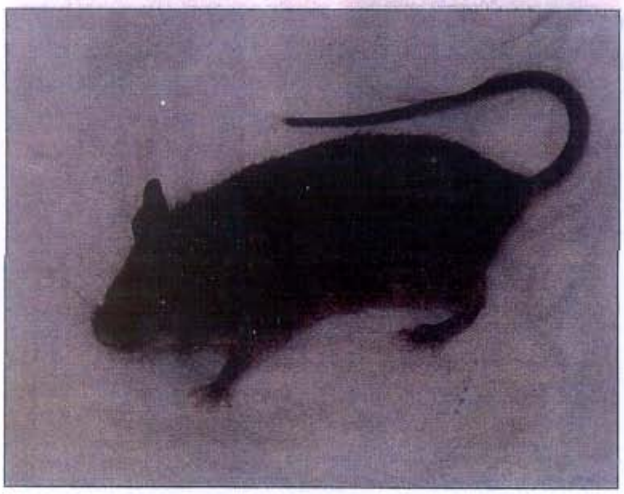
Mouse Genome Sequencing Consortium*

*A list of authors and their affiliations appears at the end of the paper

NATURE | VOL 420 | 5 DECEMBER 2002 | www.nature.com/nature

WHAT MAKES a HUMAN?

COMPARE GENOMES OF MOUSE & MAN!!



A member of the 129 strain of inbred mice commonly used in targeted mutagenesis studies.

MOUSE CHROMOSOMES

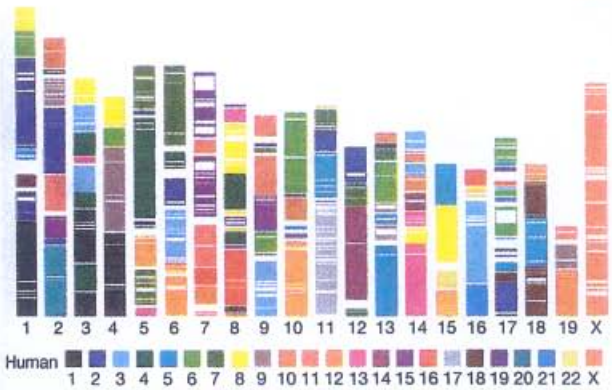


Figure 3 Segments and blocks >300 kb in size with conserved synteny in human are superimposed on the mouse genome. Each colour corresponds to a particular human chromosome. The 342 segments are separated from each other by thin, white lines within the 217 blocks of consistent colour.

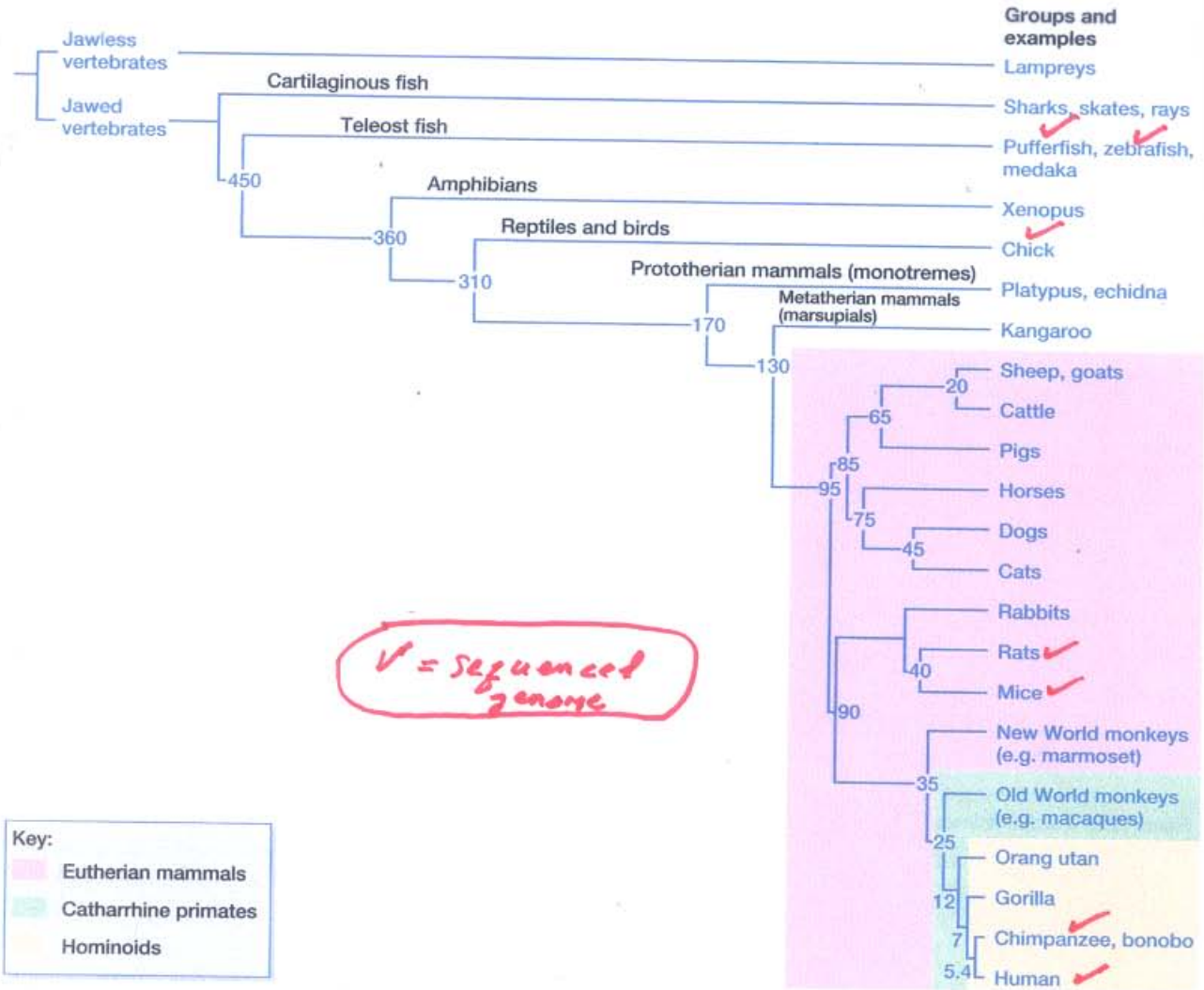
& VICE VERSA!

Table 10 **Gene count in human and mouse genomes**

Genome feature	Human		Mouse	
	Initial (Feb. 2001)	Current (Sept. 2002)	Initial* (this paper)	Extended† (this paper)
Predicted transcripts	44,860	27,048	28,097	29,201
Predicted genes	31,778	22,808	22,444	22,011
Known cDNAs	14,882	17,152	13,591	12,226
New predictions	16,896	5,656	8,853	9,785
Mean exons/transcript‡	4.2 (3)	8.7 (6)	8.2 (6)	8.4 (6)
Total predicted exons	170,211	198,889	191,290	213,562

MICE ARE POWERFUL "TOOLS" FOR STUDYING HUMAN DISEASES

VERTABRATE AND MAMMALIAN RELATIONSHIPS



V = sequenced genome

Figure 12.24: A simplified vertebrate phylogeny.

Numbers at nodes show estimated divergence times in millions of years.

COMPARATIVE GENOMICS

HUMAN & CHIMP GENOMES ARE VIRTUALLY IDENTICAL

Chimp Genome Draft Online

The relationship between humans and chimps just got a little easier to understand. This week, the consortium that has been unraveling the DNA sequence of our closest cousin for the past year put its results into the public domain. Robert Waterston of the University of Washington, Seattle, and his colleagues at the Broad Institute in Cambridge, Massachusetts (including the former genome center of the Whitehead Institute for Biomedical Research), and at the Washington University Genome Sequencing Center in St. Louis, Missouri, have determined the order of many of the 3.1 billion bases of a single male chimp's genome. Until now, only pieces of deciphered DNA were available, and their placement along the two dozen chromosomes was uncertain.

On average, each base was sequenced only four times. That's far short of the current 10-fold coverage of the human genome, but it's enough to put together a rough draft with many bases in the right order, which the researchers deposited online in GenBank. The consortium matched up the human and chimp genomes base by base as much as possible, an alignment that will make it easier for researchers to find elusive genes and regulatory regions. The matchup will also highlight specific differences between the two genomes, perhaps further hinting at what set us apart. The sequence is more than researchers could have hoped for a few years ago, says Ajit Varki of the University of California, San Diego, and it "will be most useful" for geneticists trying to find genes responsible for inherited diseases.

Work on the chimp sequence continues, but in the meantime, the consortium is taking the next few months to analyze the data it has. It expects to publish results early next year. –E.P.

CREDITS: (LEFT TO RIGHT) PAT POWERS AND CHERRY'S SCHAFER/GETTY IMAGES; C

10,000,000 YEAR Divergence

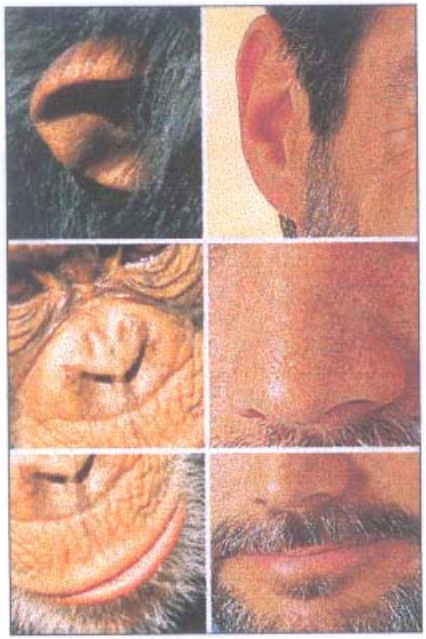
But differences should indicate why a man is a man & a chimp a chimp

Key to understanding unique Human Features at Molecular Level!!

Compare all Mammalian Genomes!

EVOLUTION

Genome Comparisons Hold Clues to Human Evolution



Hear no evil. Changes in genes for hearing, olfaction, and speech helped prompt human evolution.

WHAT IS THE OVERALL ORGANIZATION OF THE HUMAN GENOME?

TABLE 9-1 Classification of Eukaryotic DNA

Protein-coding genes
Solitary genes
Duplicated and diverged genes (functional gene families and nonfunctional pseudogenes)
Tandemly repeated genes encoding rRNA, 5S rRNA, tRNA, and histones
Repetitious DNA
Simple-sequence DNA
Moderately repeated DNA (mobile DNA elements)
Transposons
Viral retrotransposons
Long interspersed elements (LINES; nonviral retrotransposons)
Short interspersed elements (SINES; nonviral retrotransposons)
Unclassified spacer DNA

UNIQUE SEQUENCES 65%

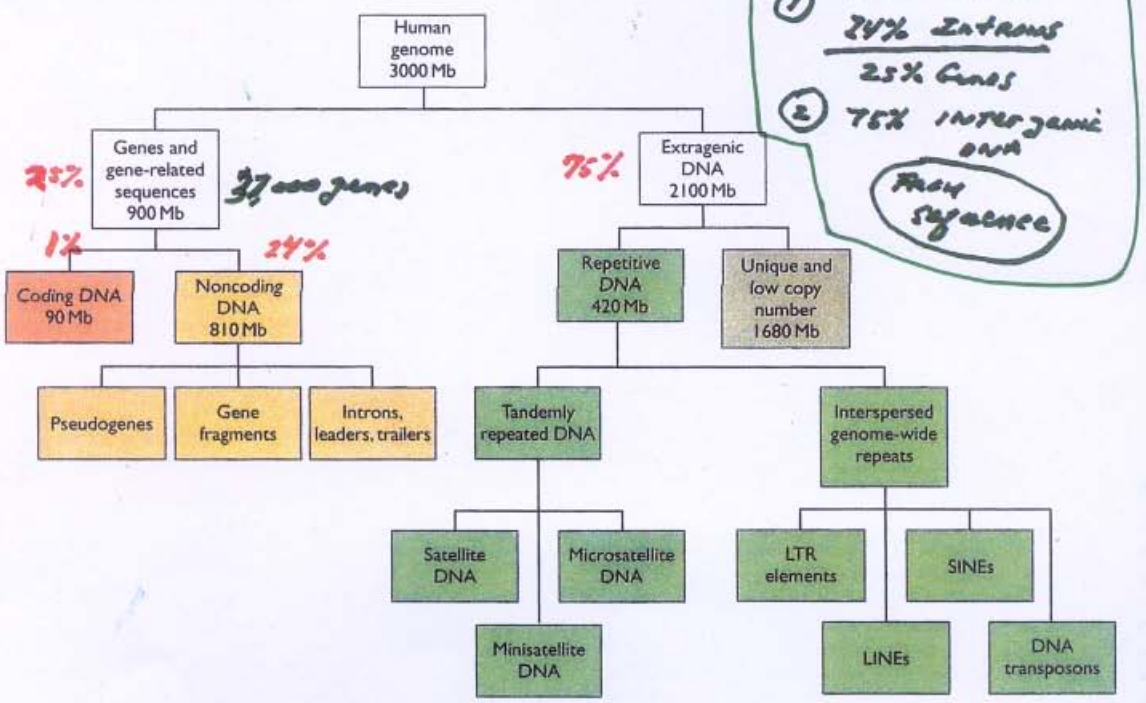
REPEATED SEQUENCES 35%

VNTR's STR's

→ Genes!
ONLY 1% Exons
24% Introns
Remaining intergenic

→ NO KNOWN FUNCTION!
Includes VNTRs!

Box 6.4: The organization of the human genome



Based on Strachan and Read (1996).

THE HUMAN GENOME CONTAINS DIFFERENT CLASSES OF REPEATED SEQUENCES

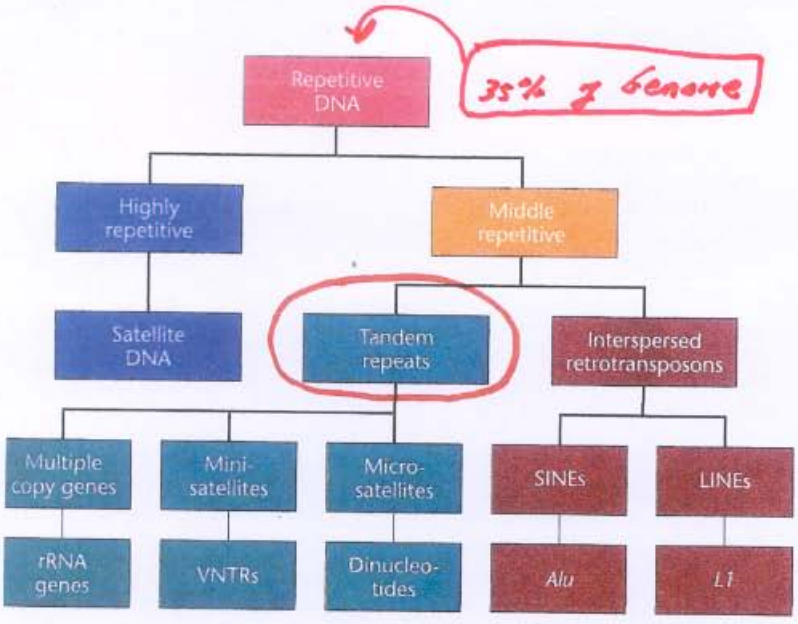


FIGURE 19.16 An overview of the various categories of repetitive DNA.

VNTRs useful for individual identity

STRs as well

Table 7.11: Major classes of tandemly repeated human DNA

USEFUL AS VNTRS

Class	Size of repeat	Major chromosomal location(s)
'Megasatellite' DNA (blocks of hundreds of kb in some cases)	several kb	Various locations on selected chromosomes
RS447	4.7 kb	~50–70 copies on 4p15 plus several copies on distal 8p
untitled	2.5 kb	~400 copies on 4q31 and 19q13
untitled	3.0 kb	~50 copies on the X chromosome
Satellite DNA (blocks often from 100 kb to several Mb in length)	5–171 bp	Especially at centromeres
α (alphoid DNA)	171 bp	Centromeric heterochromatin of all chromosomes
β (*Sau3* A family)	68 bp	Centromeric heterochromatin of 1, 9, 13, 14, 15, 21, 22 and Y
Satellite 1 (AT-rich)	25–48 bp	Centromeric heterochromatin of most chromosomes and other heterochromatic regions
Satellites 2 and 3	5 bp	Most, possibly all, chromosomes
Minisatellite DNA (blocks often within the 0.1–20 kb range)	6–64 bp	At or close to telomeres of all chromosomes
telomeric family	6 bp	All telomeres
hypervariable family	9–64 bp	All chromosomes, often near telomeres
Microsatellite DNA (blocks often less than 150 bp)	1–4 bp	Dispersed throughout all chromosomes

VNTRs STRs

HUMAN DNA SEQUENCE ORGANIZATION

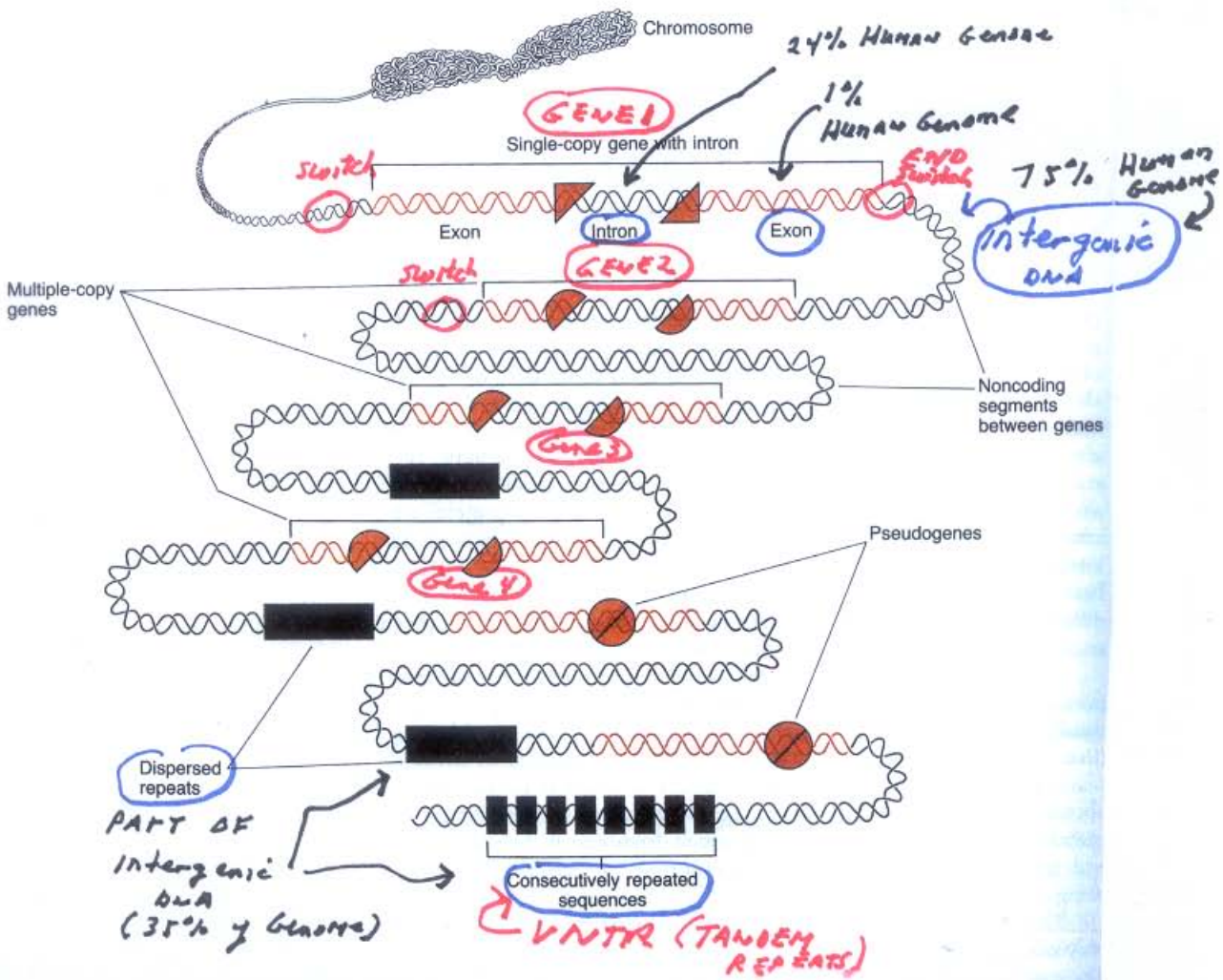


Figure 7.1 Occurrence of different kinds of unique and repeated DNA segments on chromosomal DNA.

CONTINUOUS STRETCH OF GENES + INTERGENIC REGIONS!

VNTRs Are Tandem Repeats & Give Rise to Allelic Variability

Cleavage sites conserved

Variable # Tandem Repeats

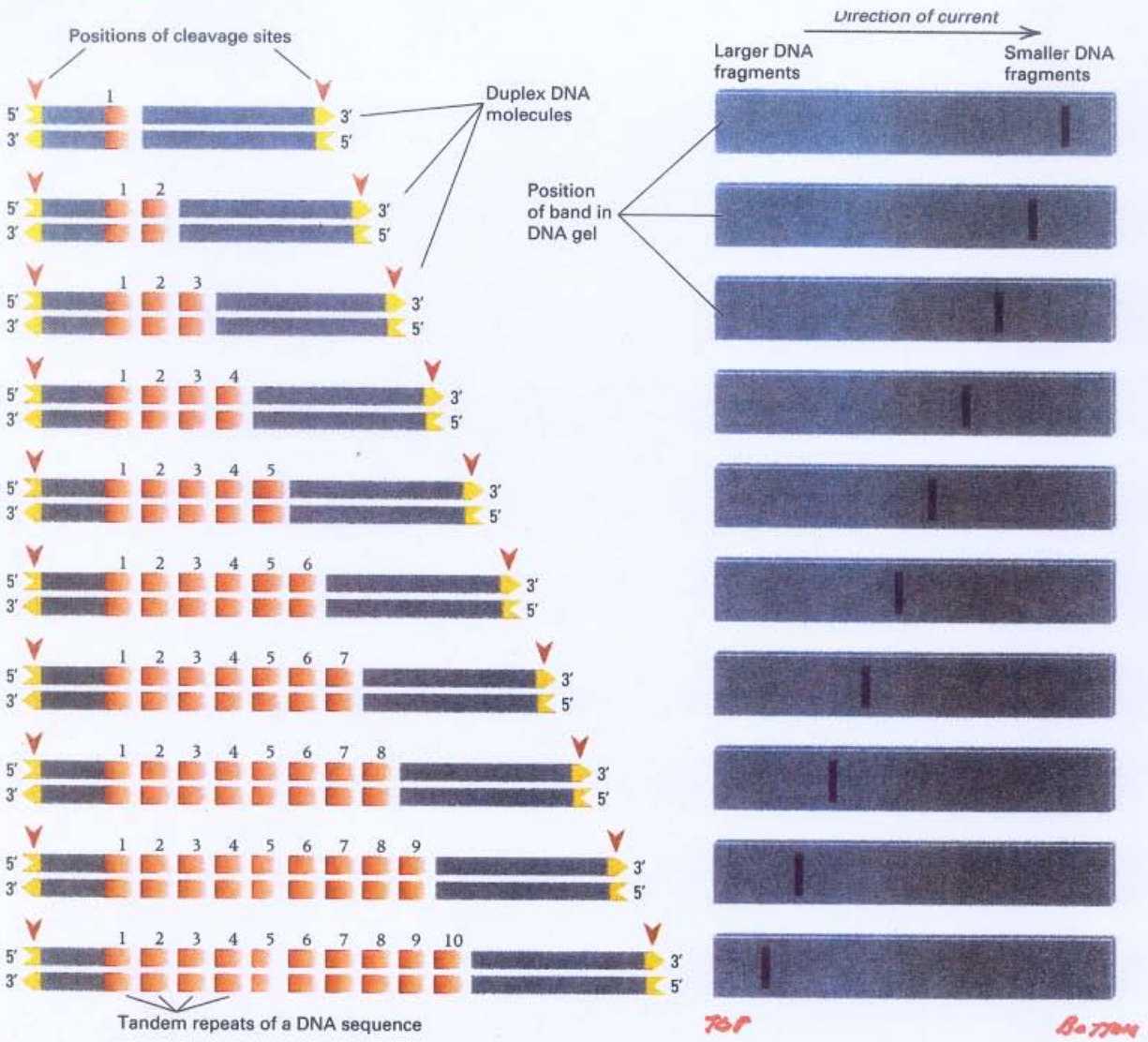


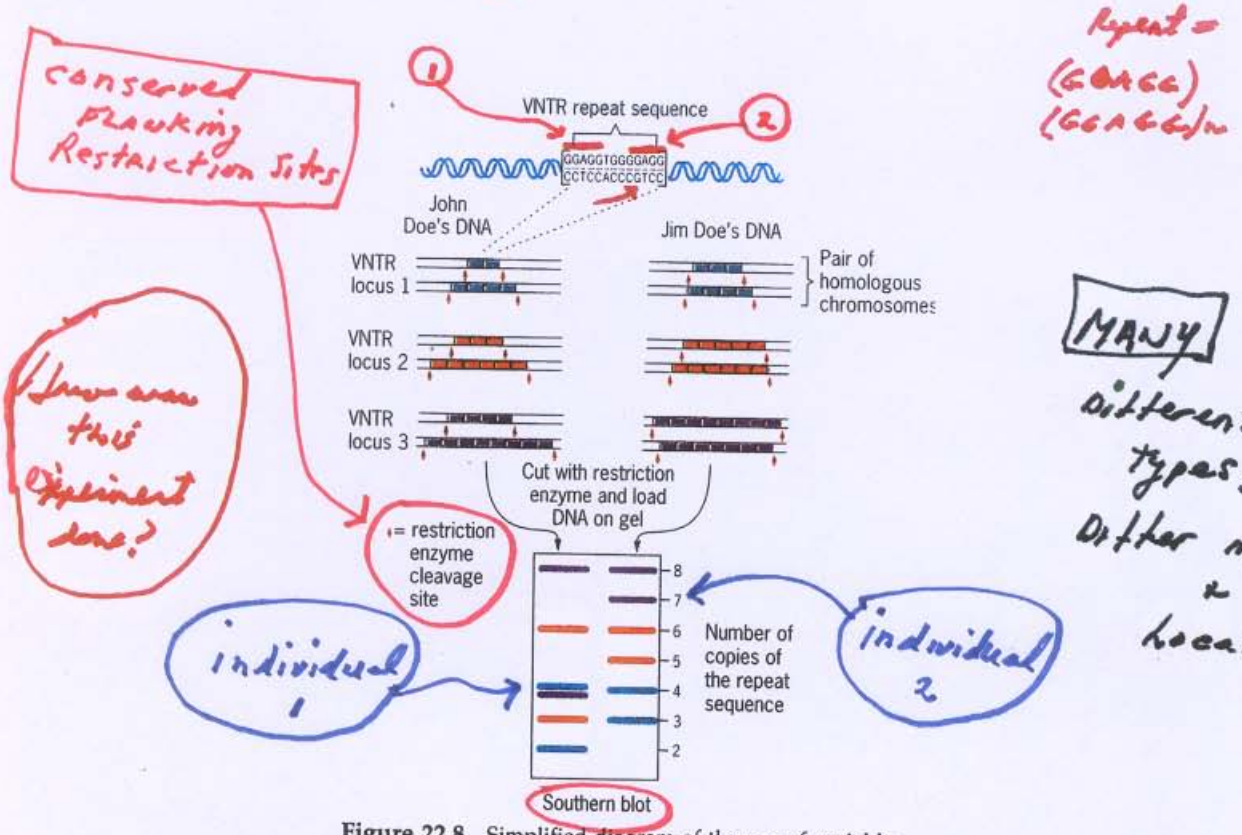
Figure 2.28 In a simple tandem repeat polymorphism (STRP), the alleles in a population differ in the number of copies of a short sequence (typically 2–60 bp) that is repeated in tandem along the DNA molecule. This example shows alleles in which the repeat number varies from 1 to 10. Cleavage at restriction sites flanking the STRP yields a unique fragment length for each allele. The alleles can also be distinguished by the size of the fragment amplified by PCR using primers that flank the STRP.

Size varies between conserved regions
Like an Accordian — AT SAME LOCUS
or CHROMOSOME Location

ANALOGOUS TO DISO FINGERPRINTS

VNTRs Are Sequence-Specific Tandem Repeats Present Throughout the Genome

BLOT METHOD



Repeat = (GGAGG) (GGAGG)n

MANY different types! Differ in Sequence & location!

Figure 22.8 Simplified diagram of the use of variable number tandem repeats in preparing DNA fingerprints.

VARY in Repeat Length (2bp - 5bp!)

2bp + 4bp!

VNTRs Generally Have Many Different Alleles at a Given Locus

Repeat = $(CA)_n$

PCR METHOD

Population of Alleles!

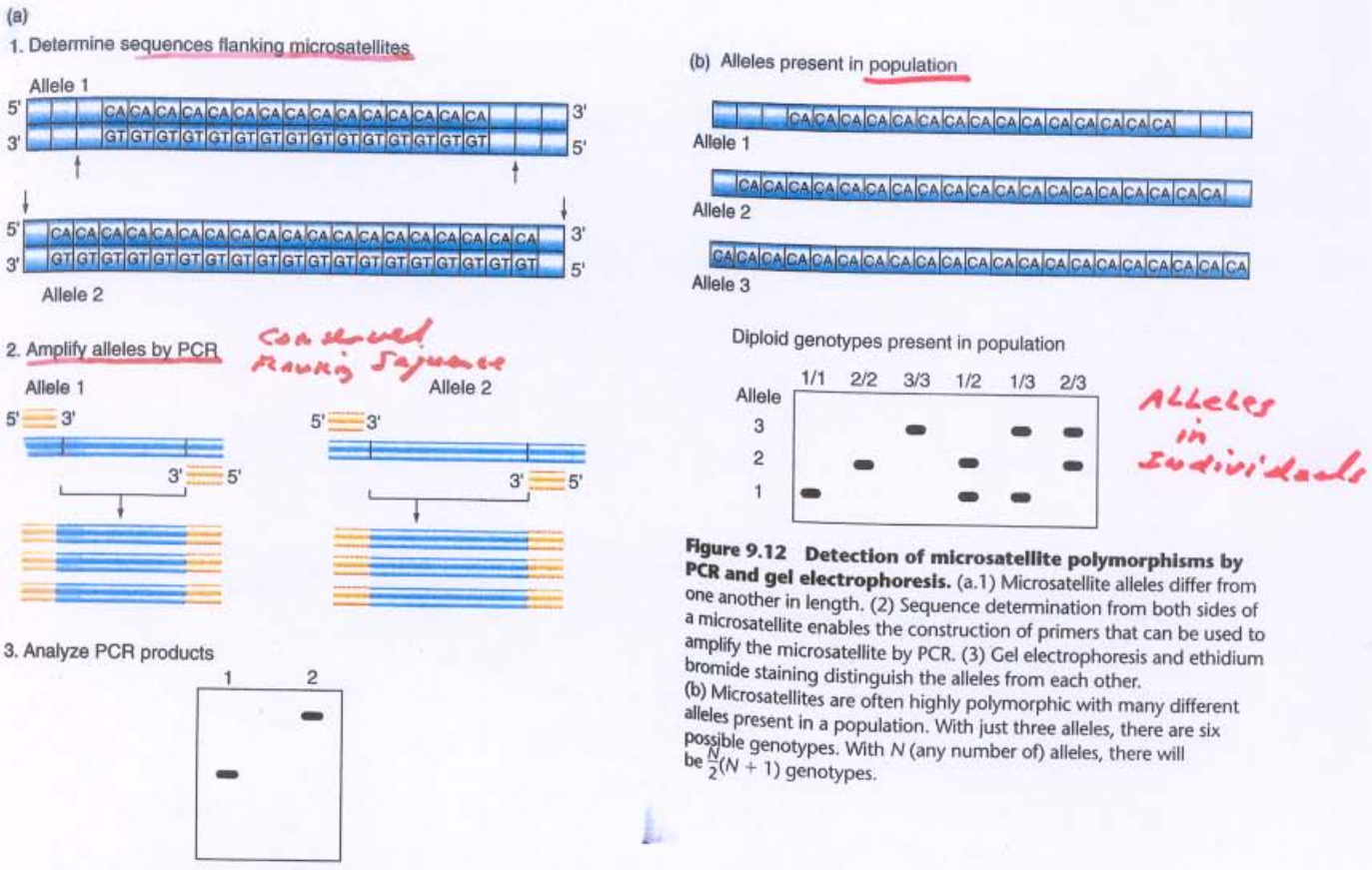


Figure 9.12 Detection of microsatellite polymorphisms by PCR and gel electrophoresis. (a.1) Microsatellite alleles differ from one another in length. (2) Sequence determination from both sides of a microsatellite enables the construction of primers that can be used to amplify the microsatellite by PCR. (3) Gel electrophoresis and ethidium bromide staining distinguish the alleles from each other.
(b) Microsatellites are often highly polymorphic with many different alleles present in a population. With just three alleles, there are six possible genotypes. With N (any number of) alleles, there will be $\frac{N}{2}(N + 1)$ genotypes.

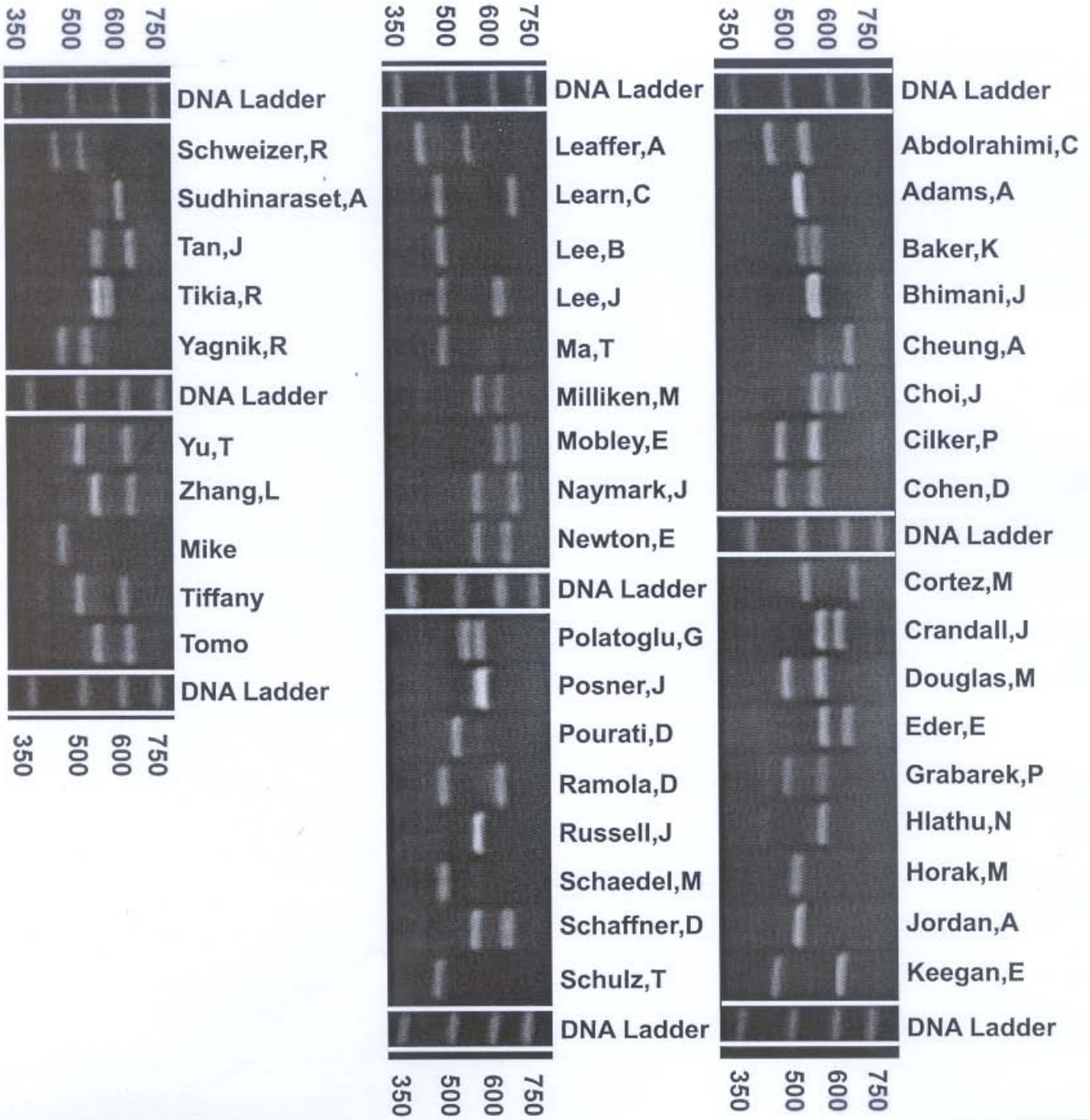
Alleles in Individuals

Useful for comparing Individuals & Populations (e.g., HCTOA) are there races?

Method Used in HCTOA Class!

DIS 80 VNTR ALLeles
in HCTOAL CLASS
W 2005

Chromosome # 1 Marker



CORE REPEAT = 16 bp
PCR Approach

VNTR D1S80 ALLeles VARy in different HUMAN Populations

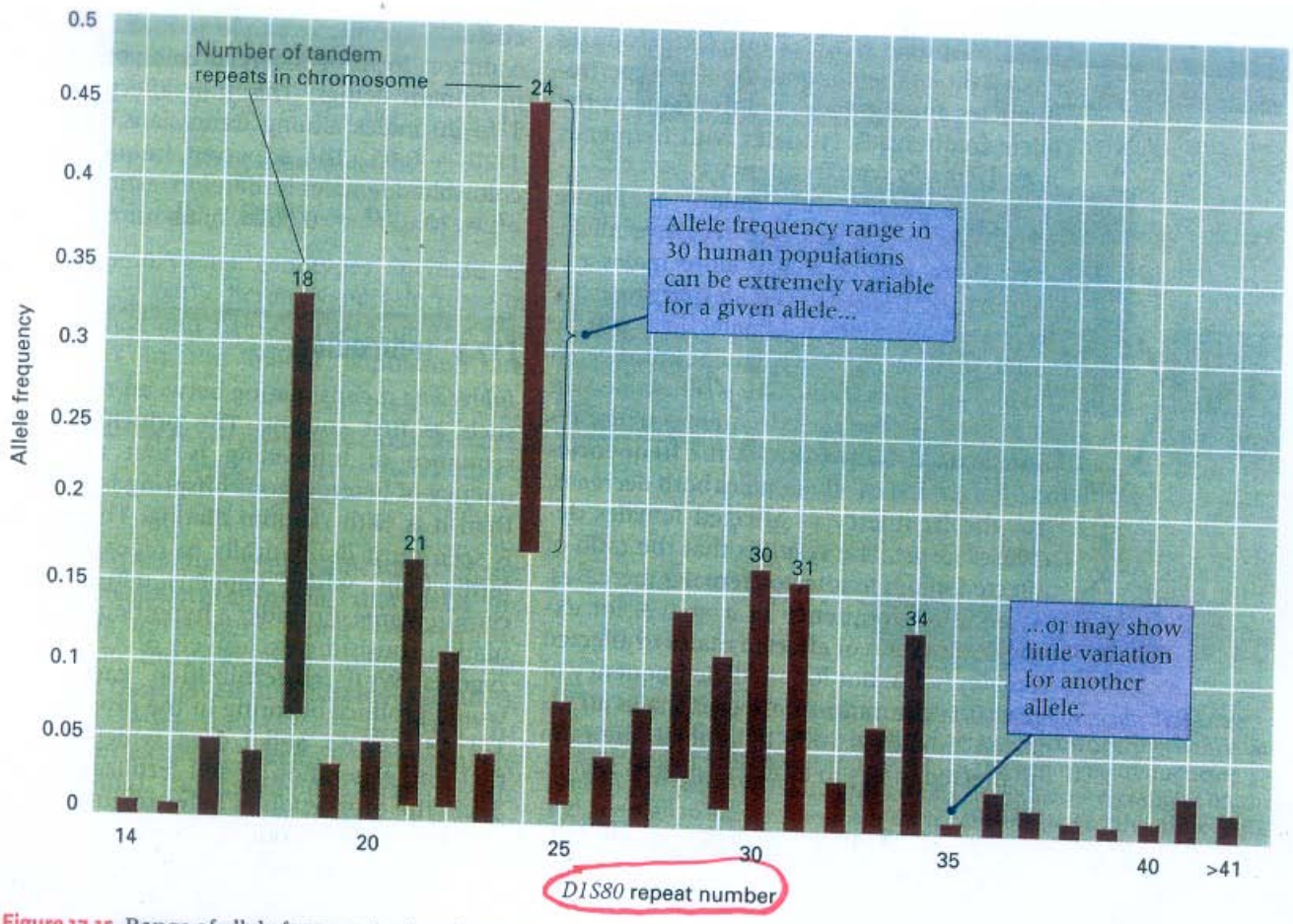
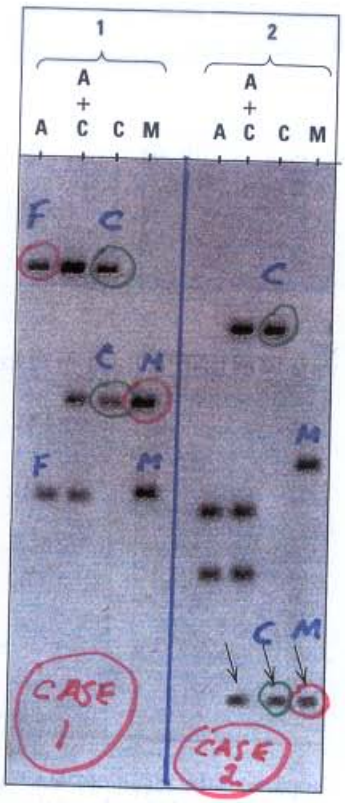


Figure 17.15 Range of allele frequencies found among human subpopulations for the VNTR *D1S80*. [Data from B. Budowle et al. *J. Forensic Science* 1995. 40:38.]

USING VNTR LOCI in PATERNITY CASES

one allele shared with child

yes A is ☺



NO A is not ☺

no allele shared with child

Figure 17.14 Use of DNA typing in paternity testing. The sets of lanes numbered 1 and 2 contain DNA samples from two different paternity cases. In each case, the lanes contain DNA fragments from the following sources: M, the mother; C, the child; A, the accused father. The lanes labeled A + C contain a mixture of DNA fragments from the accused father and the child. The arrows in case 2 point to bands of the same size that are present in lanes M, C, and A + C. Note that the male accused in case 2 could not be the father because neither of his bands is shared with the child. [Courtesy of R. W. Allen.]

RECALL --- RELATIONSHIP BETWEEN CHROMOSOMES, ALLELES, Individuals, & Populations

VNTR LOCUS

MANY VNTR alleles

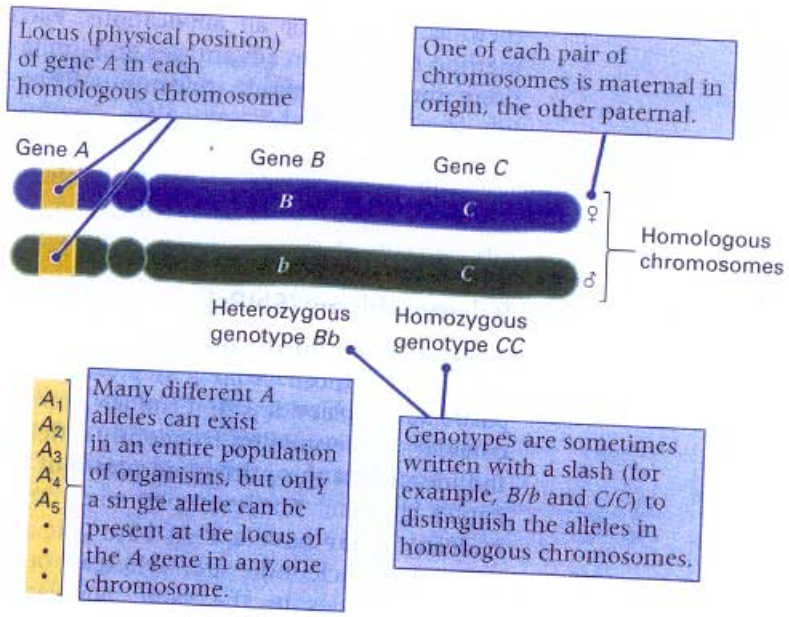
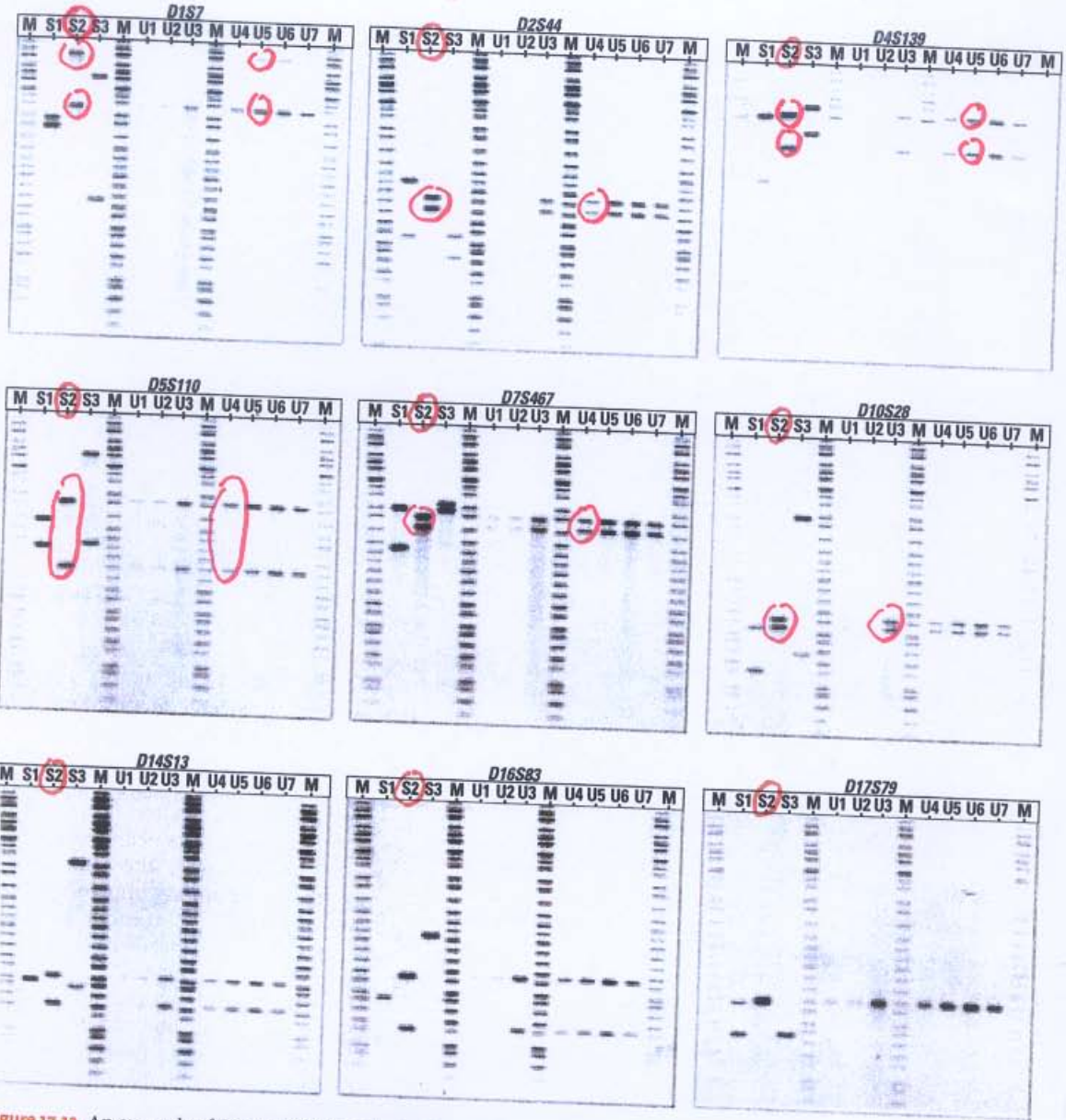


Figure 2.22 Key concepts and terms used in modern genetics. Note that a single gene can have any number of alleles in the population as a whole, but no more than two alleles can be present in any one individual.

Multiple Single-Locus VNTRs used in a Criminal Case

U = Semen from Rape Victim 7 victims



NOTE: Power of using many different VNTR markers!

Figure 17.13 An example of DNA typing in a criminal case. Each panel is the result of DNA typing for a different VNTR. The lanes marked S1, S2, and S3 contain DNA from blood samples of three male suspects; those in columns U1 through U7 contain DNA from semen samples collected from seven female victims of rape. The lanes marked M contain molecular-weight markers. In each case, the DNA from suspect S2 matches the samples obtained from the victims. [Courtesy of Steven J. Redding, Office of the Hennepin County District Attorney, Minneapolis, and Lowell C. Van Berkom and Carla J. Finis, Minnesota Bureau of Criminal Apprehension.]

Who done it!!

But also who is innocent?

ORIGINS OF VNTR VARIABILITY

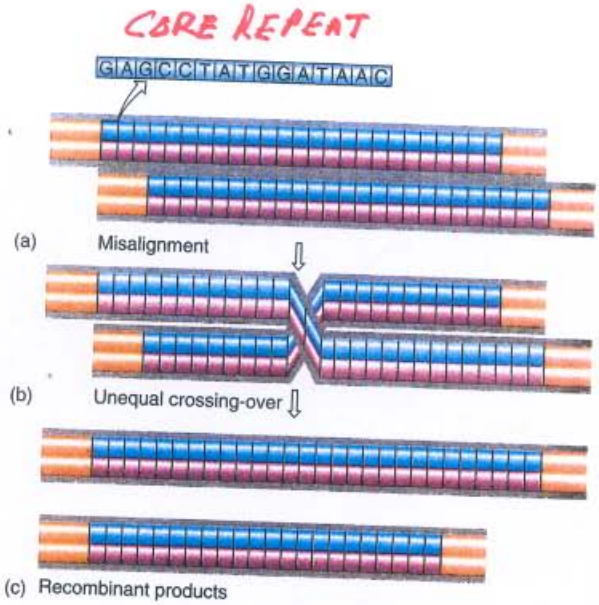
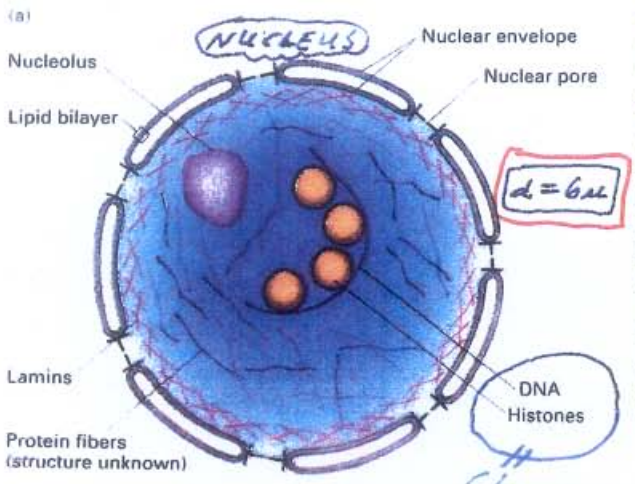


Figure 9.4 Minisatellites are highly polymorphic because of their potential for misalignment and unequal crossing-over. Minisatellites are composed of relatively long tandem repeating units of identical sequence. (a) Misalignment and (b) unequal crossing-over produce (c) recombinant products that contain different numbers of repeating units than either parental locus; each new recombinant product is a new allele.

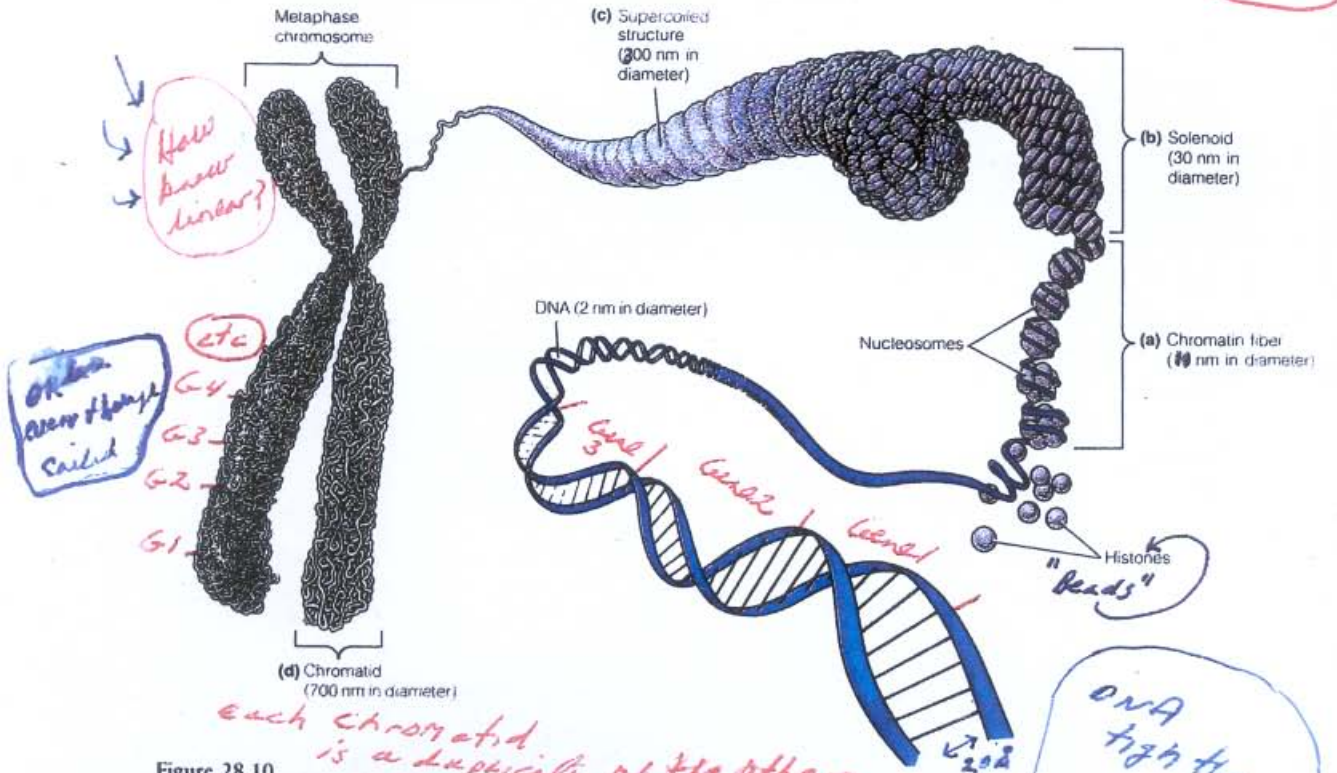
During Crossing over in Meiosis

The HUMAN GENOME IS PACKAGED INTO CHROMOSOMES



◀ **Figure 8-60** (a) Generalized diagram of eukaryotic nucleus showing identifiable structural elements. (b) Electron micrograph of nuclear pore–lamin complexes isolated from rat nuclei. Nuclear pores *(arrows)* are embedded in fibrous lamin proteins (la). (c) A transmission electron micrograph of a whole mount of a HeLa cell, showing a skeletal network within the nucleus. The cell was prepared by removing lipids and soluble factors with a mild detergent. The remaining skeletal struture was then treated to remove most of the DNA. The sample was fixed with glutaraldehyde, but no heavy-metal shadowing was done. [See S. Penman et al., 1982, *Cold Spring Harbor Symp. Quant. Biol.* **46**:1013.] *Photograph (b) courtesy of N. Dwyer. Reproduced from the* Journal of Cell Biology, *1976, by copyright permission of Rockefeller University Press. Photograph (c) courtesy of S. Penman.*

Nucleus = 6 μ diameter
DNA = 6×10^6 nm in length!



each chromatid is a duplicate of the other

Number of Genes per chromosome

DNA tightly coiled

why?

DYNAMIC / Coiling Chromosomes

Figure 28.10
Levels of chromatin structure. The beaded string structure is a 11-nm fiber, which folds into a "solenoidal" 30-nm fiber with about six nucleosomes per turn. This can further fold to form thick 200-nm fibers that can be observed in electron micrographs of chromosomes or nuclei.

HISTONE PROTEINS INTERACT WITH DNA TO MAKE A CHROMOSOME

DNA division occured?

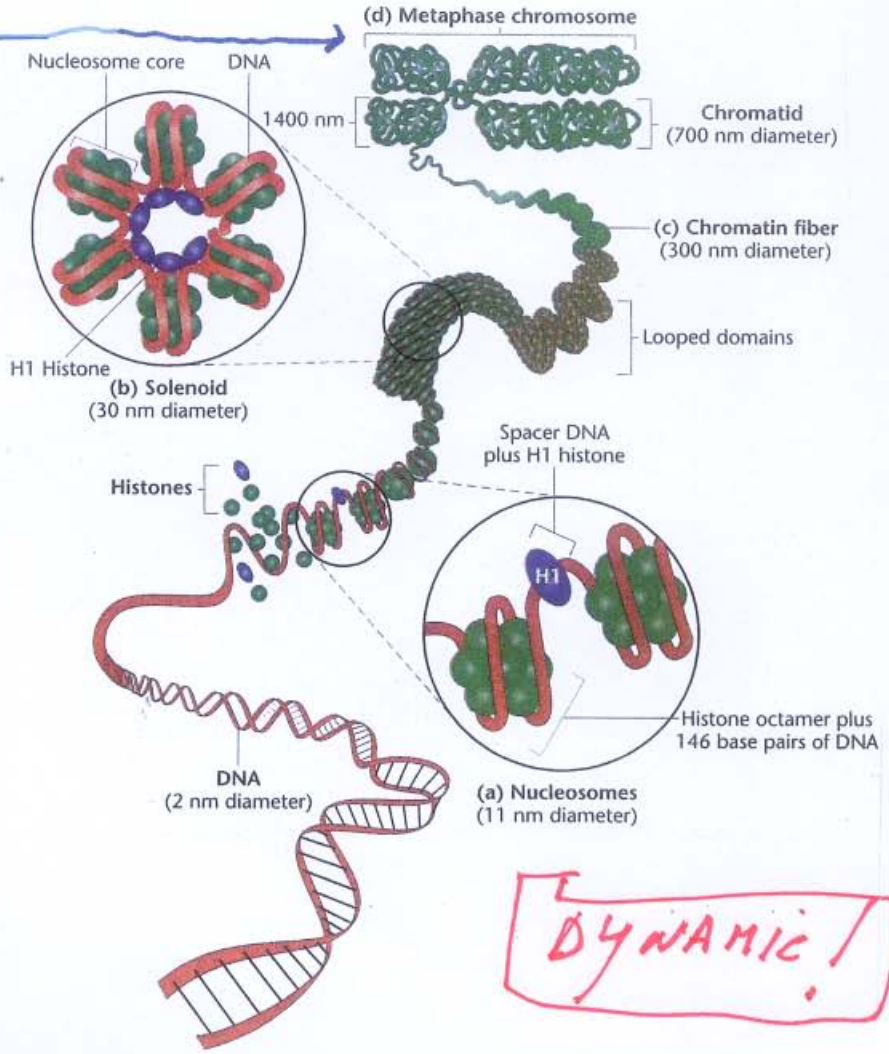


FIGURE 19.12 General model of the association of histones and DNA in the nucleosome, illustrating the way in which the chromatin fiber may be coiled into a more condensed structure, ultimately producing a mitotic chromosome. GenCDX

DYNAMIC!

Significance of coiling?

CHROMOSOMES CAN BE CHARACTERIZED USING A MICROSCOPE AND CONSTRUCTING A Karyotype

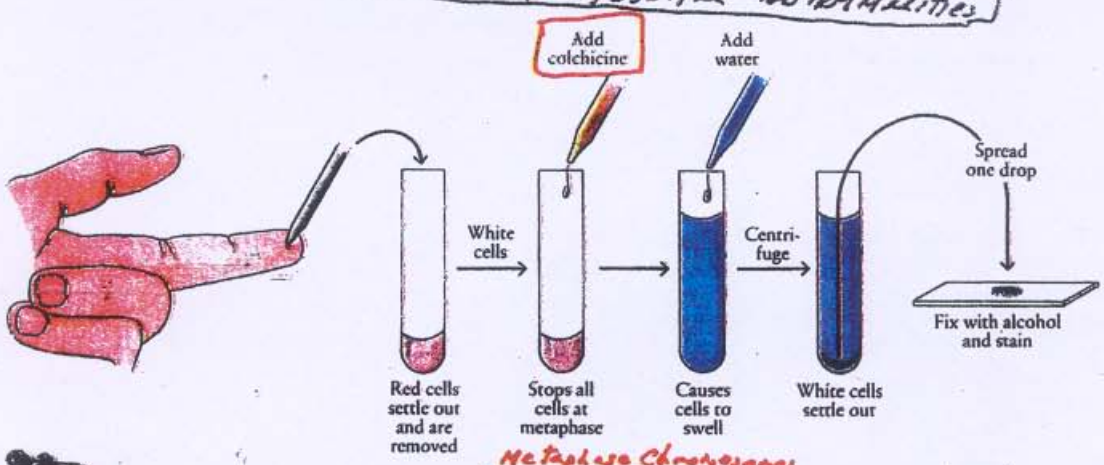
Preparation of a Karyotype

AT METAPHASE

why "short" then?

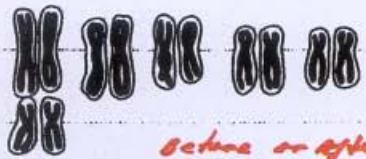
Chromosome typing for the identification of gross chromosomal abnormalities is being carried out at an increasing number of genetic counseling centers throughout the United States. The result of the procedure is a graphic display of the chromosome complement, known as a karyotype. The chromosomes shown in a karyotype are mitotic metaphase chromosomes, each consisting of two sister chromatids held together at their centromeres. To prepare a karyotype, cells in the process of dividing are interrupted at metaphase by the addition of colchicine, a drug that prevents the subsequent steps of mitosis from taking place by interfering with the spindle microtubules. After treating and staining, the chromosomes are photographed, enlarged, cut out, and arranged according to size. Chromosomes of the same size are paired according to centromere position, which results in different "arm" lengths. From the karyotype, certain abnormalities, such as an extra chromosome or piece of a chromosome, can be detected.

Karyotypes are Needed to detect Chromosomal Abnormalities



C- Metaphase Chromosomes

colchicine Metaphase!

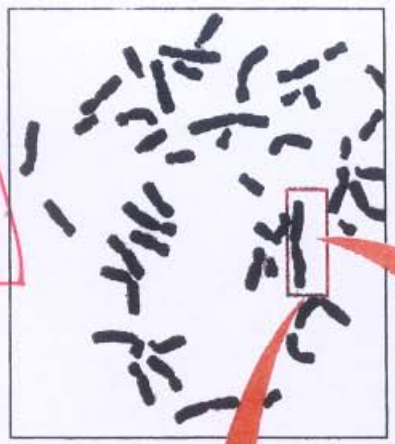


Paste in order of diminishing size with centromere on pencil line

before or after DNA replication?

2 Chromatids after DNA replication = 2 DNA molecules

CHROMOSOMES HAVE STRUCTURES That ARE VISIBLE in Light and Electron Microscopes



Light MICROSCOPE

ight micrograph of
uman chromosomes
enlarged 600 times)

SCANNING ELECTRON MICROSCOPE

Electron micrographic 3-D image
(enlarged 30,000 times)

TRANSMISSION Electron MICROSCOPE

Electron micrograph
of fixed chromosome
(enlarged 30,000 times)

Figure 1.3 Human chromosomes.

Chromatids
centromere
telomere

A chromosome during division

EACH CHROMOSOME HAS A UNIQUE MORPHOLOGY + BANDING PATTERN

22 PAIRS OF AUTOSOMES + X + Y = 24 Pieces of DNA!

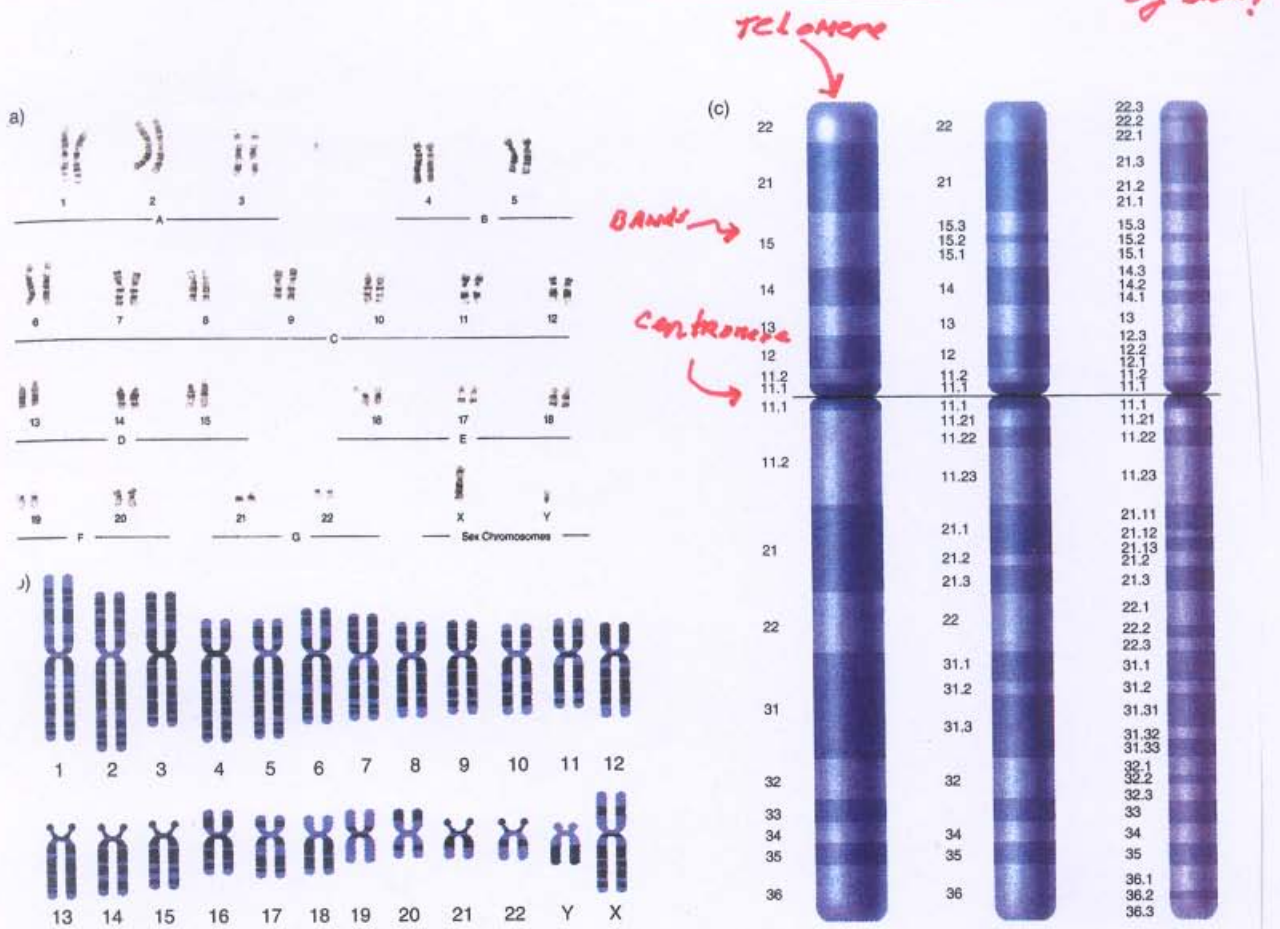


Figure 10.3 The human karyotype: Banding distinguishes the chromosomes. (a) Photograph of a complete set of human chromosomes at metaphase. Staining with Giemsa dye accentuates the bands and interbands. (b) Idiograms for the complete set of human chromosomes. An idiogram is an idealized diagram of the banding pattern associated with a stained chromosome. (c) Chromosome 7 at three different levels of banding resolution. As staining techniques improve, it becomes possible to resolve what previously appeared as a single band into a series of bands and interbands, producing more and more bands along each chromosome. Thus, at one resolution, 7q31 appears as one band. At a slightly higher resolution, 7q31 becomes two bands (7q31.1 and 7q31.3) flanking an interband (7q31.2); and at an even higher resolution, 7q31.3 itself appears as two bands (7q31.31 and 7q31.33) and an interband (7q31.32).

What causes banding patterns of chromosomes to be unique?

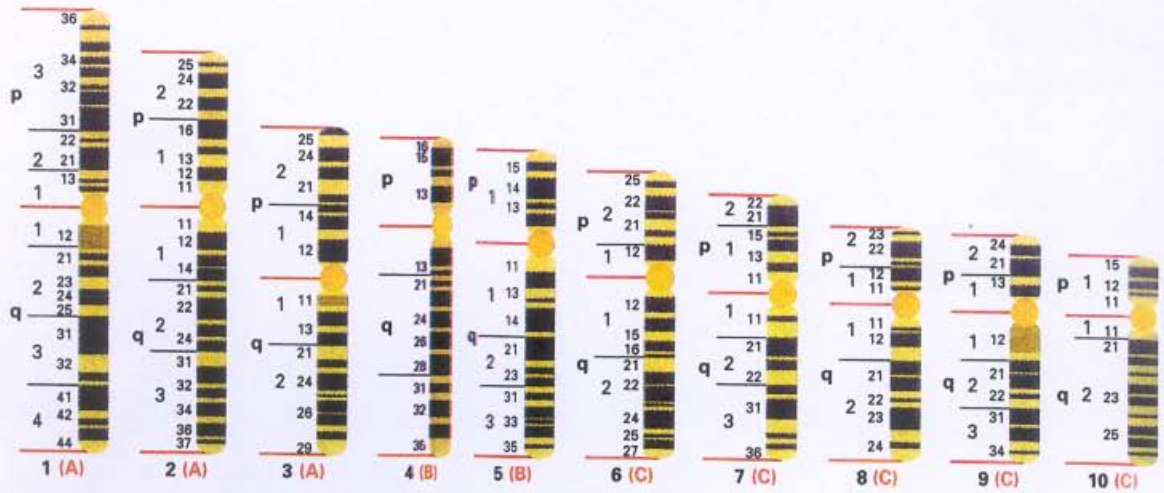
Size of bands?

SP

CHROMOSOME NOMENCLATURE

Table 9.1 Conventional karyotype symbols used in human genetics

A–G	Chromosome groups
1–22	Autosome designations
X, Y	Sex-chromosome designations
p	Short arm of chromosome
q	Long arm of chromosome
ter	Terminal portion: pter refers to terminal portion of short arm, qter to terminal portion of long arm
+	Preceding a chromosome designation, indicates that the chromosome or arm is extra; following a designation, indicates that the chromosome or arm is larger than normal
−	Preceding a chromosome designation, indicates that the chromosome or arm is missing; following a designation, indicates that the chromosome or arm is smaller than normal
mos	Mosaic
/	Separates karyotypes of clones in mosaics—e.g., 47, XXX/45,X
dup	Duplication
dir dup	Direct duplication
inv dup	Inverted duplication
del	Deletion
inv	Inversion
t	translocation
rcp	Reciprocal translocation
rob	Robertsonian translocation
r	Ring chromosome
i	Isochromosome (two identical arms attached to a single centromere, like an attached-X chromosome in *Drosophila*)



BANDING PATTERNS CAN BE USED TO DISTINGUISH CHROMOSOMES & LOCATE GENES

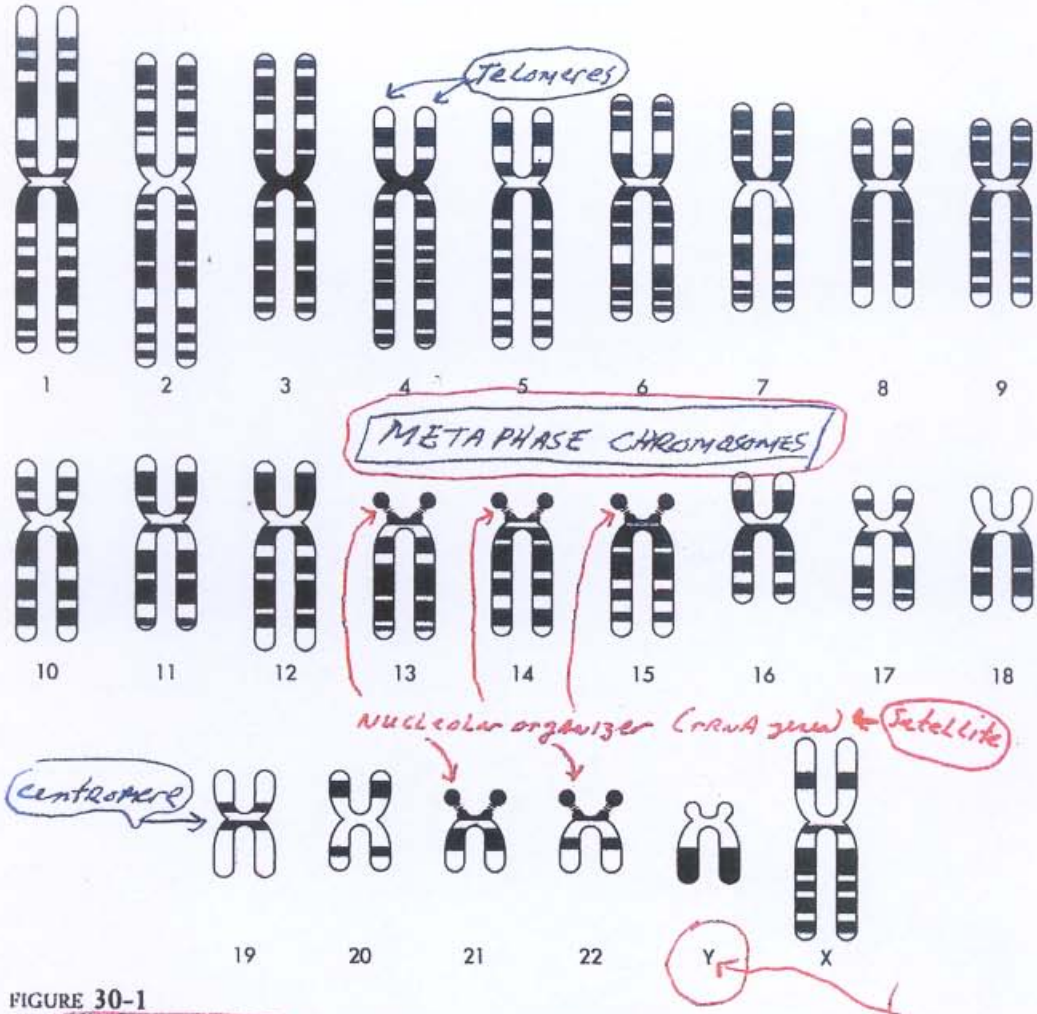
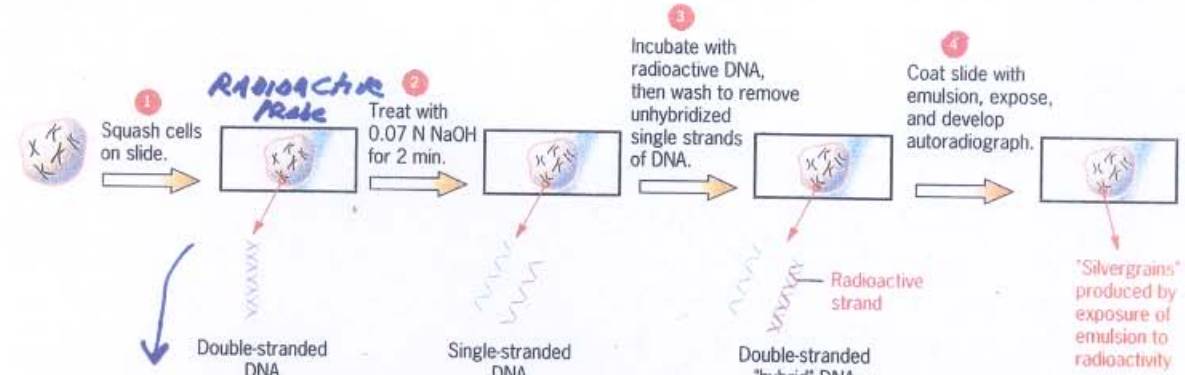


FIGURE 30-1
The haploid human genome. This is a schematic drawing of 1 of each of the 23 human chromosomes, showing the pattern of staining seen with the Giemsa banding method. Chromosomes are first treated with trypsin and then stained with Giemsa. The patterns of light and dark bands are characteristic for each chromosome; and translocations, deletions, and other structural abnormalities can be identified. Typically 400 bands can be seen per haploid genome, and each band represents on average 7.5×10^6 bp, or twice as many base pairs as in the entire *E. coli* genome! Chromosome 1 constitutes 8.4 percent, and the Y chromosome about 2.0 percent, of the human genome. Taking the *E. coli* genome as a unit of genome size, a cytogenetic band is 2 genome units, and the Y chromosome is 15 genome units.

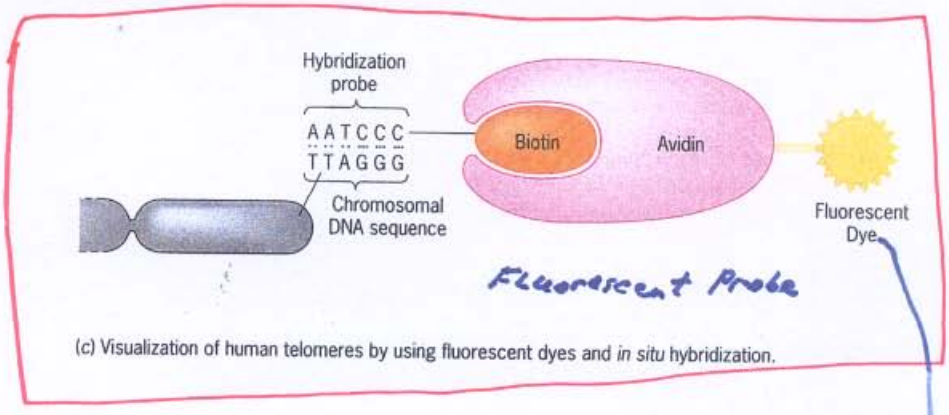
Ave band size = 7.5×10^6 bp or 7.5×10^6 bp
larger than size of E. coli Genome!

IN SITU HYBRIDIZATION WITH FLUORESCENT PROBES CAN IDENTIFY GENES & CHROMOSOMES



(a) Steps in performing *in situ* hybridization.

(b) Autoradiograph showing chromosomal locations of mouse satellite DNA sequences.



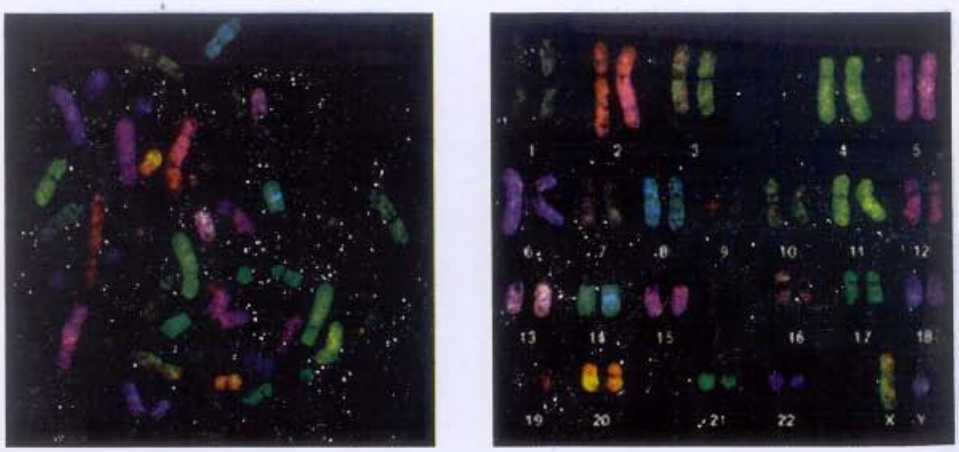
(c) Visualization of human telomeres by using fluorescent dyes and *in situ* hybridization.

Figure 11.15 Localization of repeated DNA sequences in chromosomes by *in situ* hybridization performed with radioactive probes (*a* and *b*) or fluorescent probes (*c* and *d*). The *in situ* hybridization procedure developed by Pardue and Gall is shown in (*a*), and one of their autoradiographs demonstrating the presence of the mouse satellite DNA sequence in centromeric heterochromatin is shown in (*b*). Use of fluorescent dyes to localize the TTAGGG repeat sequence to the telomeres of human chromosomes is illustrated in (*c*), and a photomicrograph demonstrating its telomeric location is shown in (*d*).

Visible Color in Microscope @ specific wavelength

HUMAN CHROMOSOMES CAN ALSO BE DISTINGUISHED BY THEIR SEQUENCES

How are these chromosomes "painted"?



(A) (B)

Figure 9.1 Human chromosome painting, in which each pair of chromosomes is labeled by hybridization with a different fluorescent probe. (A) Metaphase spread showing the chromosomes in a random arrangement as they were squashed onto the slide. (B) A karyotype, in which the chomosomes have been grouped in pairs and arranged in conventional order. Chromosomes 1–20 are arranged in order of decreasing size, but for historical reasons, chromosome 21 precedes chromosome 22, even though chromosome 21 is smaller. [Courtesy of Johannes Wienberg and Thomas Ried.]

Table 7.2: DNA content of human chromosomes[a]

Chromosome	Amount of DNA (Mb)	Chromosome	Amount of DNA (Mb)
1	263	13	114
2	255	14	109
3	214	15	106
4	203	16	98
5	194	17	92
6	183	18	85
7	171	19	67
8	155	20	72
9	145	21	50
10	144	22	56
11	144	X	164
12	143	Y	59

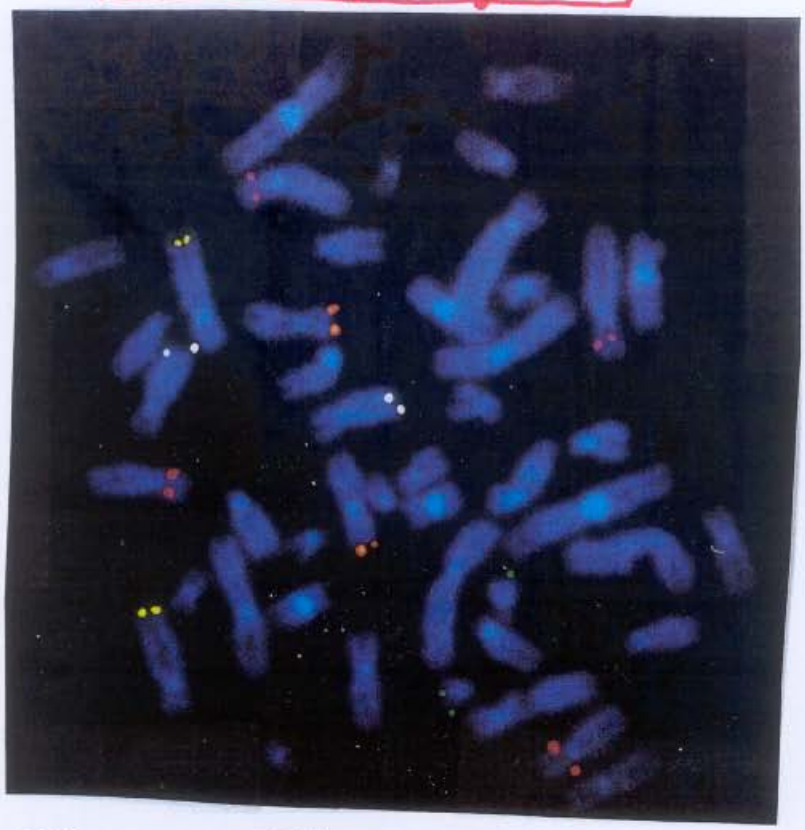
[a] The DNA content is given for chromosomes prior to entering the S (DNA replication) phase of cell division (see *Figure 2.2*). Data abstracted from electronic reference 1.

AND AMOUNT OF DNA!

FROM SEQUENCE DATA!

In SITU Hybridization

MAPPING GENES TO CHROMOSOMES AND SPECIFIC REGIONS



How correlate gene to chromosome position + band?

Pre-Sequence Approach

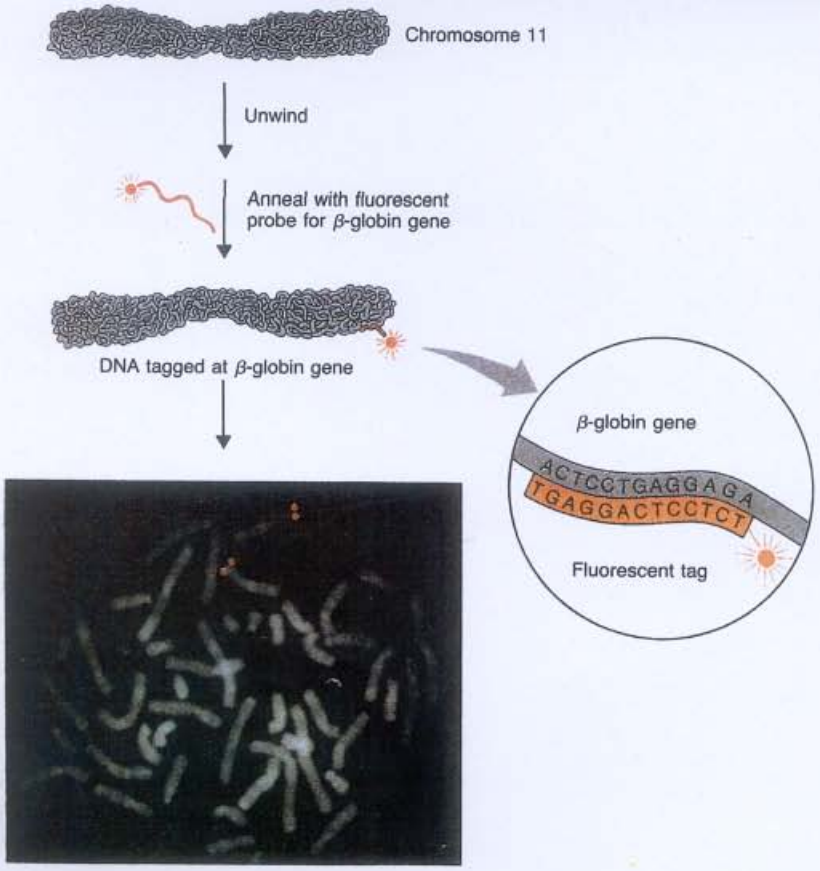


Figure 7.5 Locating the position of the β-globin gene on human chromosome 11.

GENES CAN BE MAPPED TO SPECIFIC BANDS OF EACH CHROMOSOME

X Chromosome

How Locate these Genes if no Probe or Sequence?

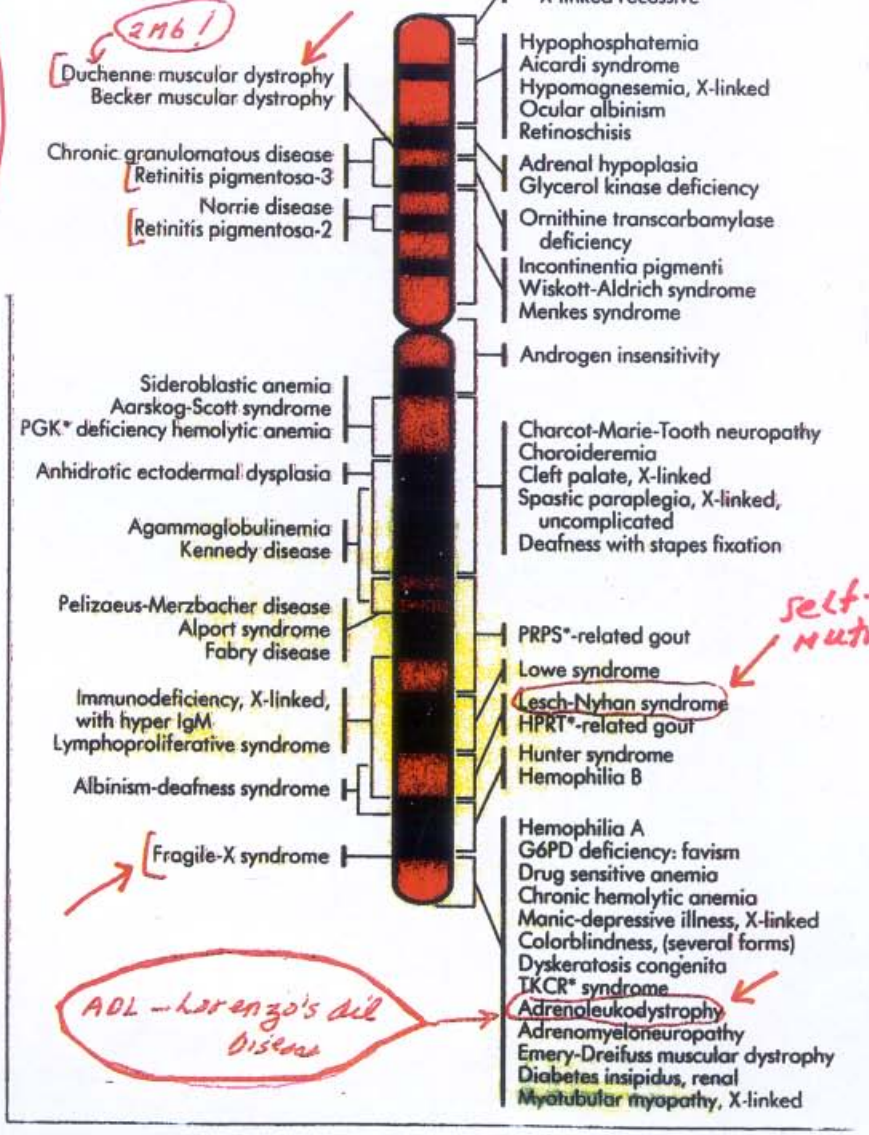
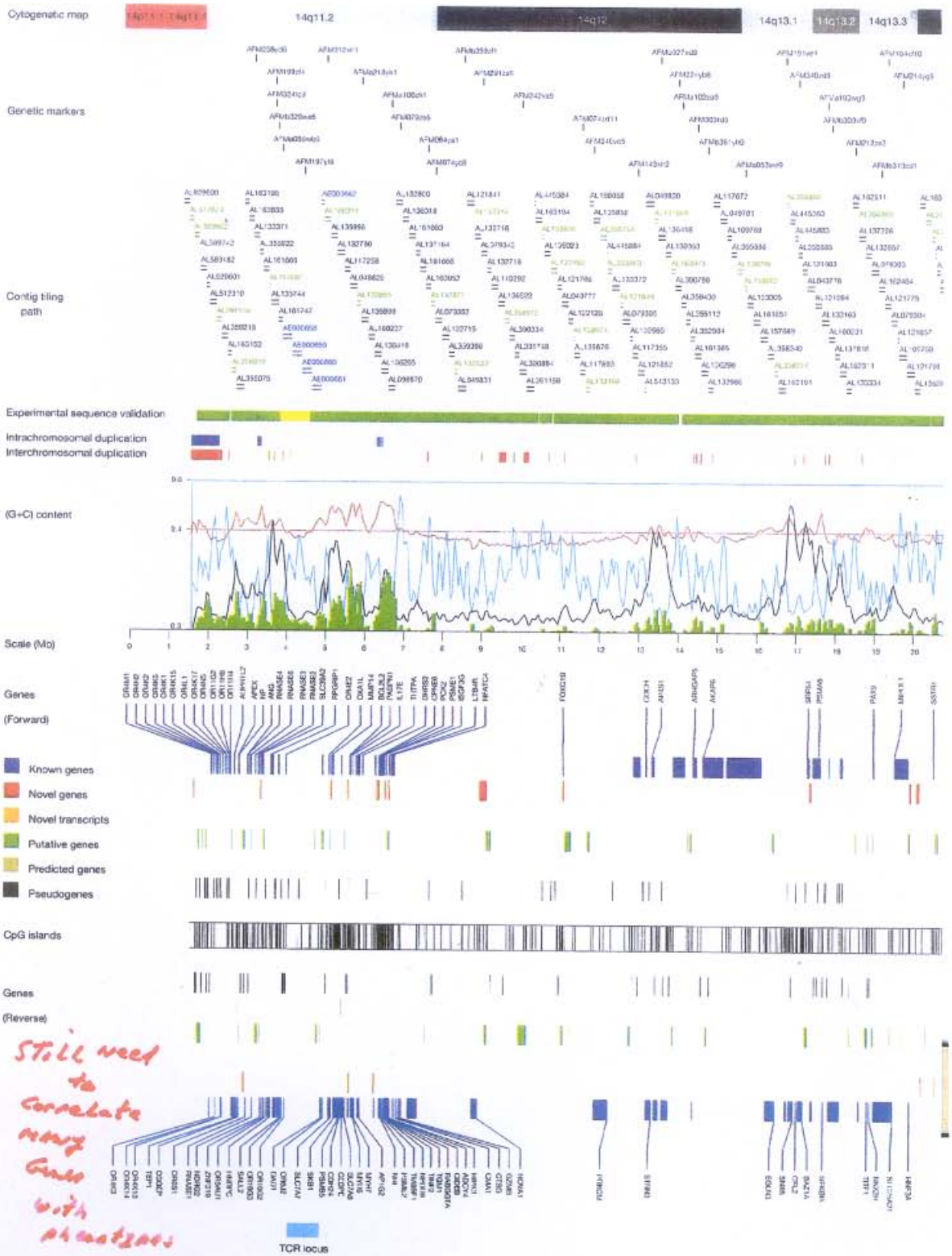


FIGURE 12-22
The human X-chromosome gene map. Over 59 diseases have now been traced to specific segments of the X-chromosome. Many of these disorders are also influenced by genes on other chromosomes. *KEY: *PGK*, phosphoglycerate kinase; *PRPS*, phosphoribosyl pyrophosphate synthetase; *HPRT*, hypoxanthine phosphoribosyl transferase; *TKCR*, torticollis, keloids, cryptorchidism, and renal dysplasia

This TASK is NOW COMPLETE WITH THE COMPLETION OF THE HUMAN GENOME SEQUENCE



Still need to correlate many Genes with phenotypes

DISEASE GENES CAN BE LOCALIZED TO SPECIFIC CHROMOSOMES

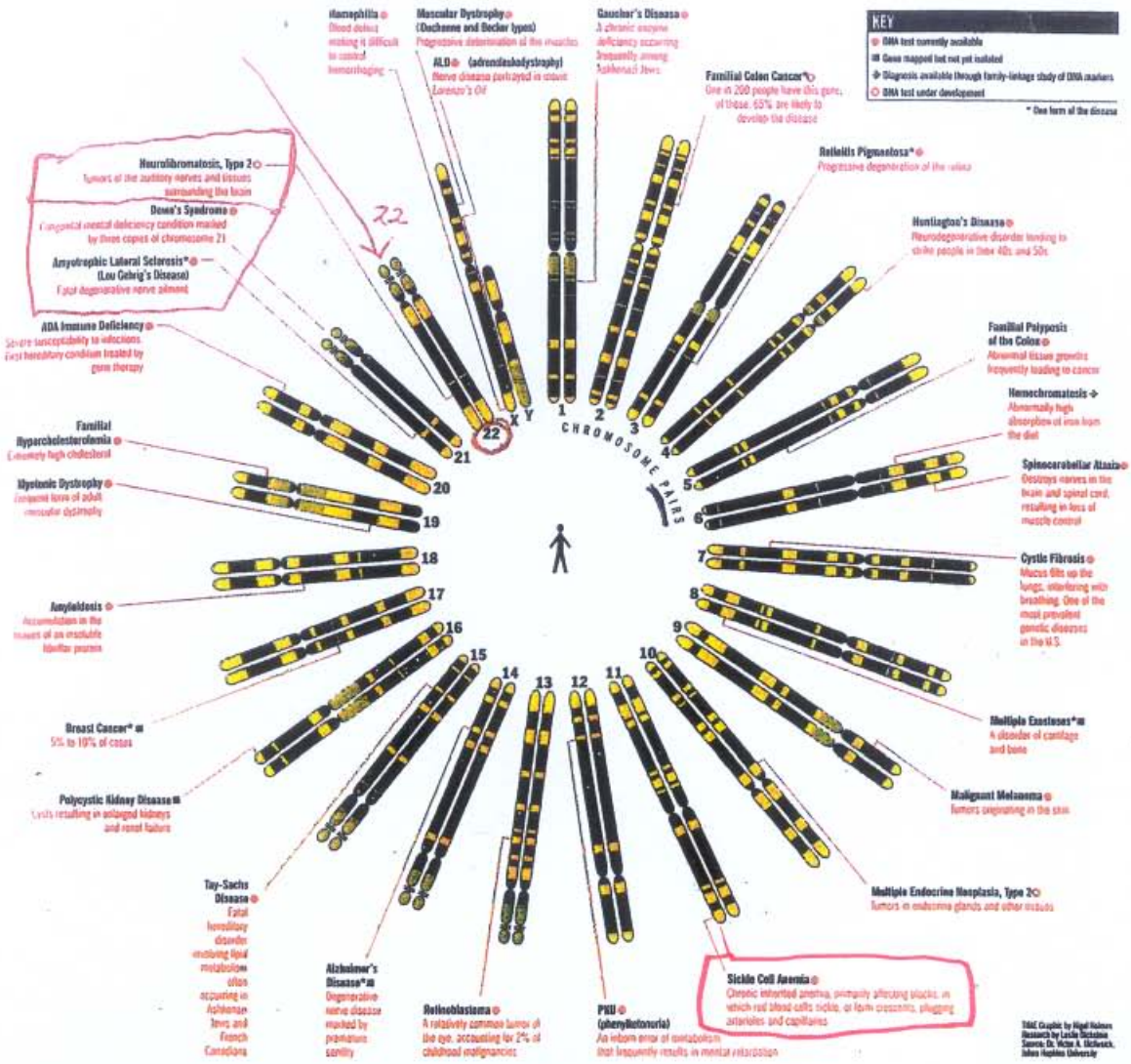


Figure 1-6 The 23 chromosomes of a human being, showing the positions of genes whose abnormal forms cause some of the better-known hereditary diseases. (*Time*)

OMIM™

BUT MANY MORE NEED TO BE CORRELATED WITH DISEASE! WHY DIFFICULT TO DO?

HOW CAN CHANGES OCCUR IN THE HUMAN GENOME?

LARGE GROSS CHANGES

TABLE 12.1 Chromosomal Rearrangements and Changes in Chromosome Number (or Ploidy).

How detect?

Chromosomal Rearrangements

	Before	After
Deletion: Removal of a segment of DNA	1 2 3 4 5 6 7 8	1 2 3 5 6 7 8
Duplication: Increase in the number of copies of a chromosomal region	1 2 3 4 5 6 7 8	1 2 3 2 3 4 5 6 7 8
Inversion: Half-circle rotation of a chromosomal region	1 2 3 4 5 6 7 8 (180° Rotation)	1 4 3 2 5 6 7 8
Translocations:		
Nonreciprocal: Unequal exchanges between nonhomologous chromosomes	1 2 3 4 5 6 7 8 / 12 13 14 15 16 17 18	12 13 4 5 6 7 8 / 14 15 16 17 18
Reciprocal: Parts of two nonhomologous chromosomes trade places	1 2 3 4 5 6 7 8 / 12 13 14 15 16 17 18	12 13 14 15 5 6 7 8 / 1 2 3 4 16 17 18
Transposition: Movement of short DNA segments from one position in the genome to another	1 2 3 4 5 6 7 8	1 2 4 5 6 3 7 8

1 Chromosomal rearrangements → large changes in DNA

How detect?

How + when would these occur?

Changes in Chromosome Number or Ploidy

Euploidy: Cells that contain only complete sets of chromosomes

Diploidy ($2x$): Two copies of each homolog — 2N ← Somatic cells

Monoploidy (x): One copy of each homolog — 1N ← Gametes

2 polyploidy → > 2n # of chromosome sets

Polyploidy: More than the normal diploid number of chromosome sets

Triploidy ($3x$): Three copies of each homolog — 3N

Tetraploidy ($4x$): Four copies of each homolog — 4N

3 Aneuploidy → change in one or more chromosome #

Aneuploidy: Loss or gain of one or more chromosomes producing a chromosome number that is not an exact multiple of the haploid number

Monosomy ($2n - 1$) MONOSOMIC — − Chromosome 3

Trisomy ($2n + 1$) TRISOMIC — + Chromosome 3

Tetrasomy ($2n + 2$) TETRASOMIC — + 2 Chromosome 3

Note that it is more accurate to denote monoploids, triploids, and tetraploids as multiples of *x*, which represents the number of different chromosomes in a complete set, rather than as multiples of *n*, the number of chromosomes in the gametes. In this table, as throughout the chapter, nonhomologous chromosomes are drawn in different colors. Different shades of the same color highlight different regions of the same chromosome.

↳ Lead to phenotypic changes / embryo death

ORIGINS OF LETHAL POLYPLOID Zygotes / Embryos

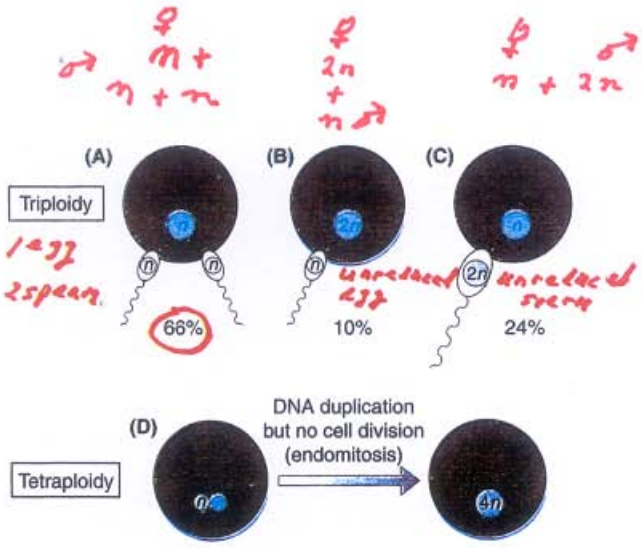


Figure 2.19: Origins of triploidy and tetraploidy.

About two-thirds of human triploids arise by fertilization of a single egg by two sperm (**A**). Other causes are a diploid egg (**B**) or sperm (**C**). Most human triploids abort spontaneously; very rarely they survive to term, but not beyond. Tetraploidy (**D**) results from failure of the first mitotic division after fertilization, and is incompatible with development.

What causes lethality with xtra genes/chromosomes?

What are the consequences of XtRA Chromosomes & Chromosome Sets?

How are these changes detected?

HUMAN GENETICS SIDELIGHT

Amniocentesis and Chorionic Biopsy: Procedures to Detect Aneuploidy in Human Fetuses

The Andersons, a couple living in Minneapolis, were expecting their first baby. Neither Donald nor Laura Anderson knew of any genetic abnormalities in their families, but because of Laura's age—38—they decided to have the fetus checked for aneuploidy.

Laura's physician performed a procedure called **amniocentesis.** A small amount of fluid was removed from the cavity surrounding the developing fetus by inserting a needle into Laura's abdomen (Figure 1). This cavity, called the amnionic sac, is enclosed by a membrane. To prevent discomfort during the procedure, Laura was given a local anesthetic. The needle was guided into position by following an ultrasound scan, and some of the amnionic fluid was drawn out. Because this fluid contains nucleated cells sloughed off from the fetus, it is possible to determine the fetus's karyotype (Figure 2). Usually the fetal cells are purified from the amnionic fluid by centrifugation, and then the cells are cultured for several days to a few weeks. Cytological analysis of these cells will reveal if the fetus is aneuploid. Additional tests may be performed on the fluid recovered from the amnionic sac to detect other sorts of abnormalities, including neural tube defects and some kinds of mutations. The results of all these tests may take up to three weeks. In Laura's case, no abnormalities of any sort were detected, and 20 weeks after the amniocentesis, she gave birth to a healthy baby girl.

Chorionic biopsy provides another way of detecting chromosomal abnormalities in the fetus. The chorion is a fetal membrane that interdigitates with the uterine wall, eventually forming the placenta. The minute chorionic projections into the uterine tissue are called *villi* (singular, villus). At 10–11 weeks of gestation, before the placenta has developed, a sample of chorionic villi can be obtained by passing a hollow plastic tube into the uterus through the cervix. This tube can be guided by an ultrasound scan, and when it is in place, a tiny bit of material can be drawn up into the tube by aspiration. The recovered material usually consists of a mixture of maternal and fetal tissue. After these tissues are separated by dissection, the fetal cells can be analyzed for chromosome abnormalities.

Chorionic biopsy can be performed earlier than amniocentesis (10–11 weeks gestation versus 14–16 weeks), but it is not as reliable. In addition, it seems to be associated with a slightly greater chance of miscarriage than amniocentesis, perhaps 2 to 3 percent. For these reasons, it tends to be used only in pregnancies where there is a strong reason to expect a genetic abnormality. In routine pregnancies, such as Laura Anderson's, amniocentesis is the preferred procedure.

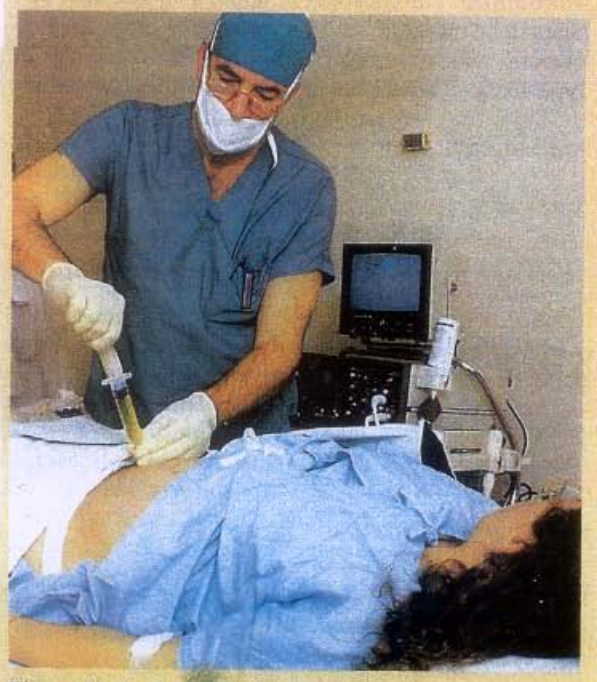


Figure 1 A physician taking a sample of fluid from the amniotic sac of a pregnant woman for prenatal diagnosis of a chromosomal or biochemical abnormality.

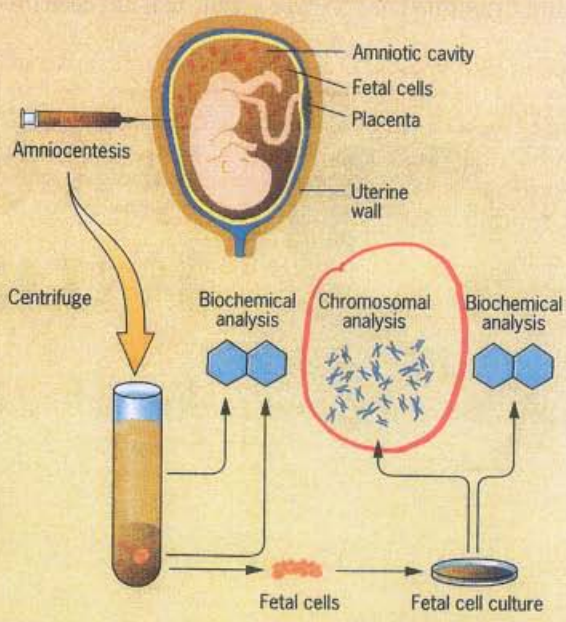


Figure 2 Amniocentesis and procedures for prenatal diagnosis of chromosomal and biochemical abnormalities.

PRENATAL Detection OF CHROMOSOMAL ABNORMALITIES

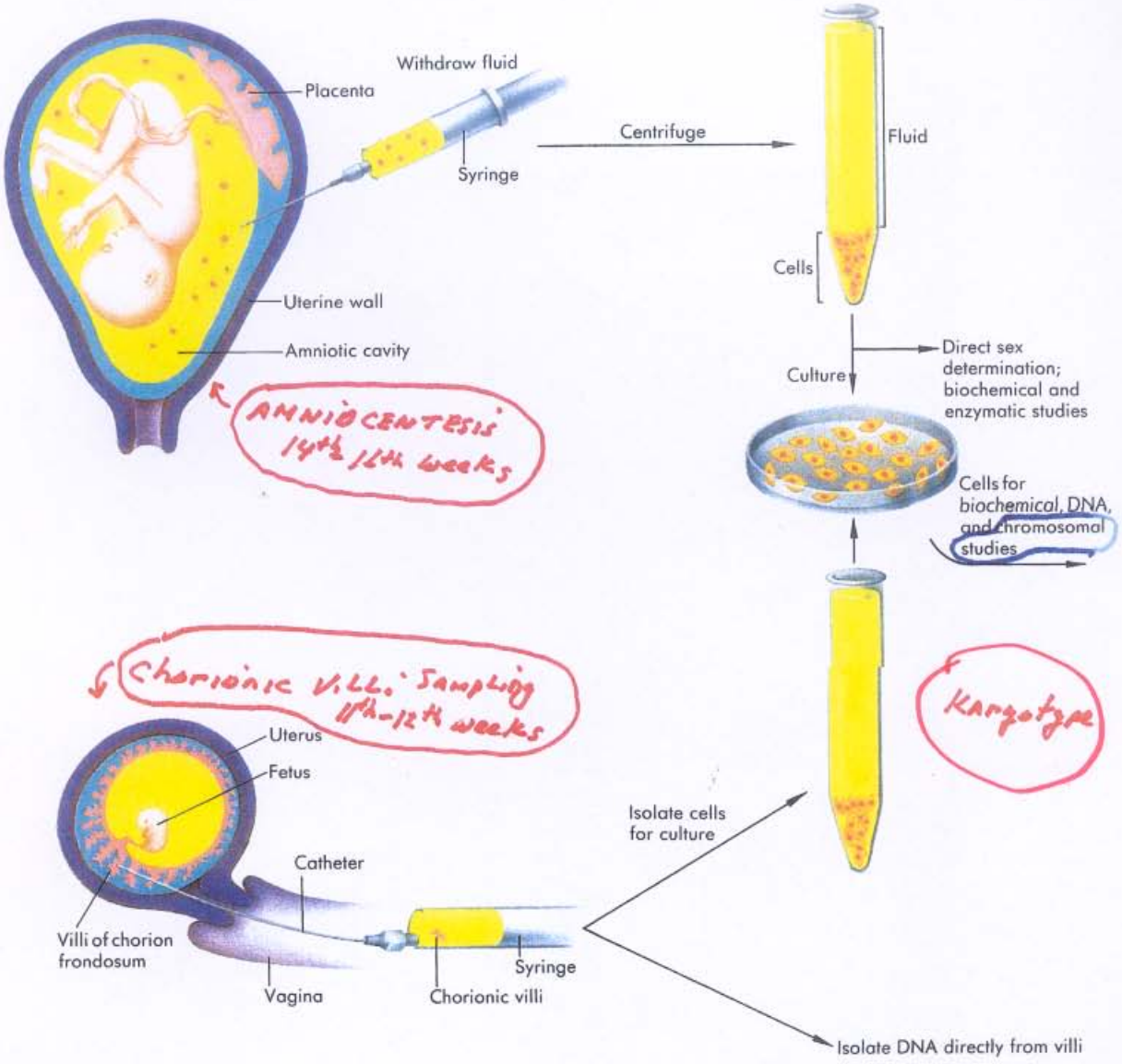
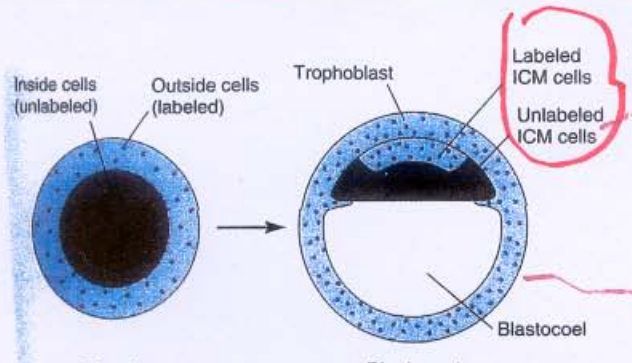


FIGURE 27-1
Amniocentesis and chorionic villus sampling. (a) A sample of amniotic fluid (mostly fetal urine and other secretions) is taken by inserting a needle into the amniotic cavity during or around the sixteenth week of gestation. The fetal cells are separated from the fluid by centrifugation. The cells can be used immediately, or more usually they are cultured so that a number of biochemical, enzymatic, and chromosomal analyses can be made. The cultured cells can also be a source of DNA. (b) Chorionic villus sampling is performed between the eighth and twelfth weeks of gestation. A catheter is introduced through the vagina or transabdominally, and a small sample of chorionic villi is drawn into the syringe. DNA can be isolated directly from the tissue, or cell cultures can be established. Note that the various elements of this figure are not drawn to scale.

ALSO CAN BE USED FOR DNA Testing

RFLP or SNP analysis

HUMAN EMBRYO FORMATION



Number of inside cells of 16-cell stage	Proportion of labeled cells in ICM
2	> 65%
5 (mean)	~ 25%
7	< 5%

Embryo/Fetus
Stem cells
Embryo/Yolk, Chorion
Chorion villi Sampling (embryonic)

Figure 5.13 Stepwise formation of the inner cell mass (ICM) in mammalian embryos. Most of the ICM cells are derived from those cells that are in an inside position at the morula stage. Thus, after selectively labeling cells on the outside of a morula, most ICM cells of the developing blastocyst are unlabeled. However, in embryos that have few inside morula cells, additional ICM cells are generated by differential cleavage of outside morula cells.

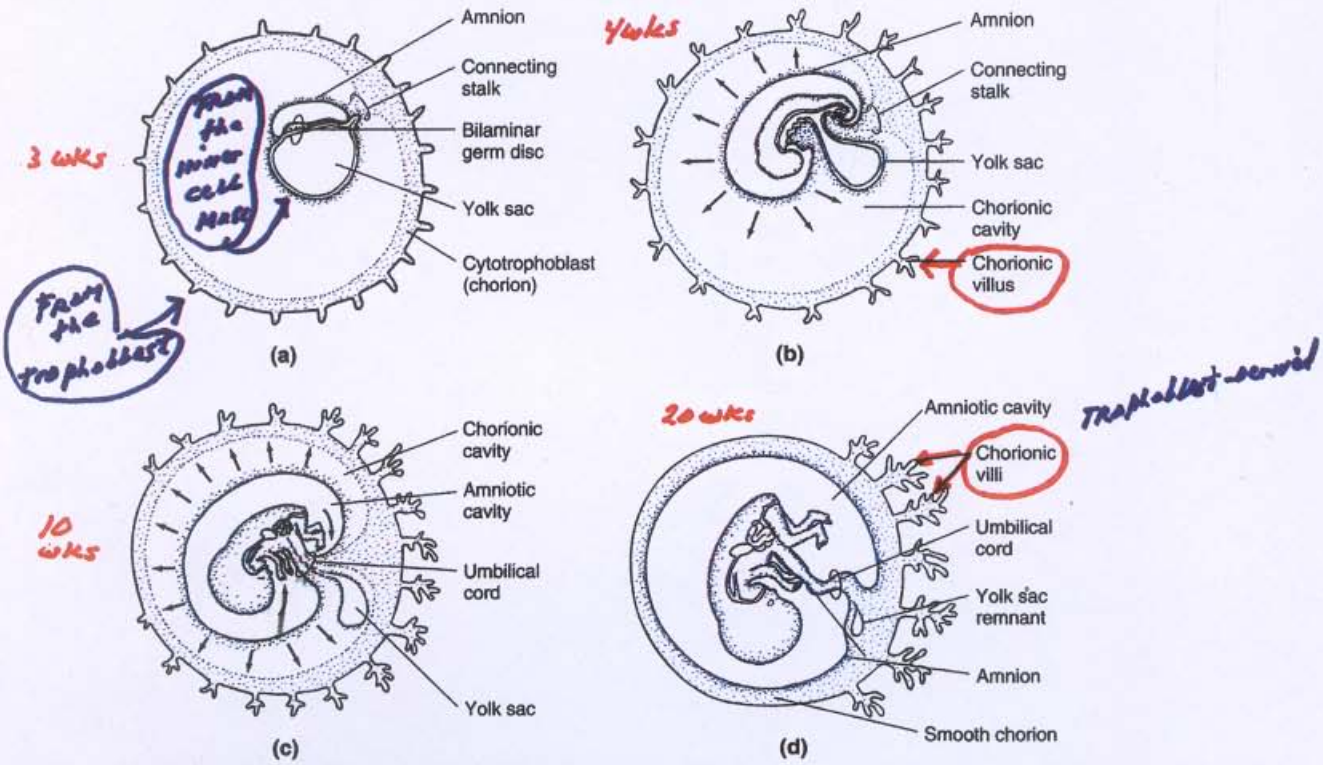


Figure 14.38 Extraembryonic membranes in human development: **(a)** at 3 weeks; **(b)** at 4 weeks; **(c)** at 10 weeks; **(d)** at 20 weeks. The connecting stalk develops into the umbilical cord. The amniotic cavity expands (arrows) until it completely fills the chorionic cavity and envelops the umbilical cord plus the remnant of the yolk sac. The chorionic villi near the umbilical cord branch and form the embryonic portion of the placenta. The other villi disappear.

PREIMPLANTATION GENE DIAGNOSIS USING PCR - DEFECTIVE GENES

USE TO ASSAY GENES (NOT) LARGE CHROMOSOME Alterations

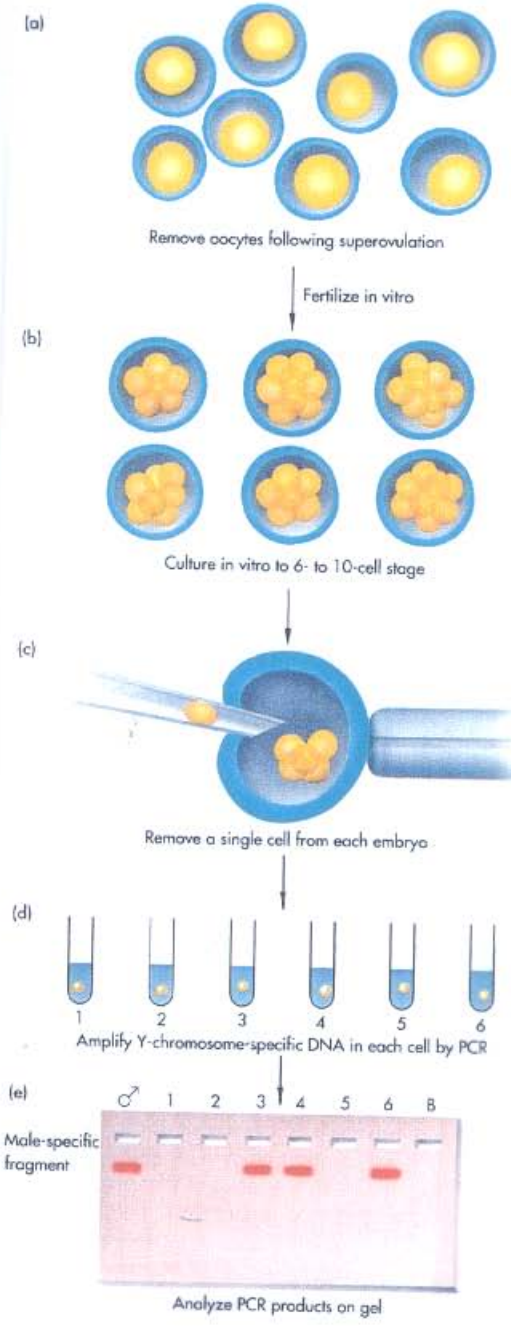
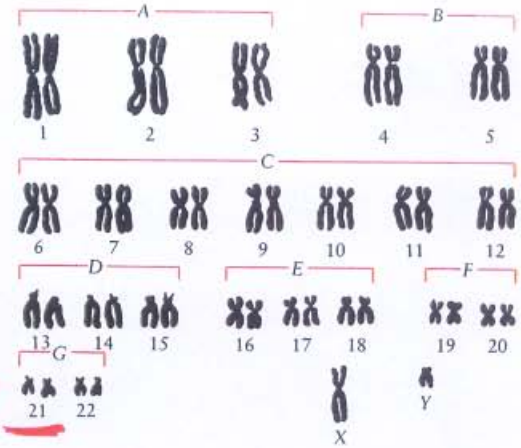


FIGURE 6-11
Determining sex of fetuses at risk for X-linked inherited disorders. (a) Oocytes are removed from the mother following superovulation and fertilized in vitro. (b) The oocytes that are fertilized successfully are cultured in vitro until there are 6 to 10 cells in each embryo. (c) A hole is made in the zona pellucida and a single cell removed from each embryo. (d) Amplification of the DYZ1 sequence is attempted. (e) Only in DNA from males is the male-specific DYZ1 sequence amplified by PCR, giving rise to a 149-bp, male-specific fragment. The lane marked with the male symbol is a positive control showing the expected fragment; the lane marked B (for "Blank") is from a PCR that included all the reagents but no DNA and is used to detect any contamination. Female embryos are negative (lanes 1, 2, and 5) and are implanted into the mothers.

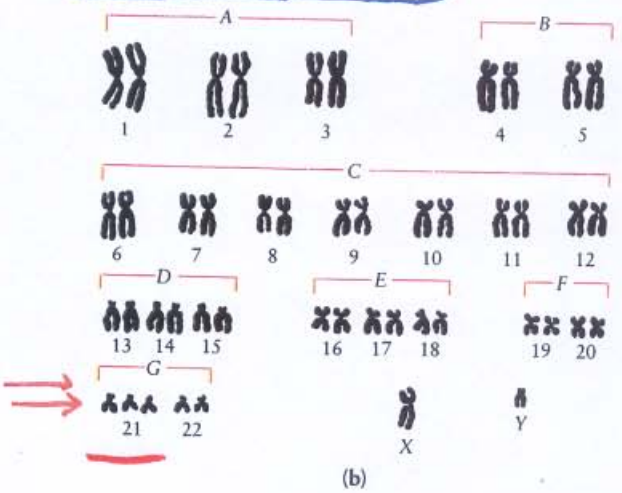
Karyotypes Reveal Chromosomal Abnormalities

19–2 *The normal diploid chromosome number of a human being is 46, 22 pairs of autosomes and two sex chromosomes. The autosomes are grouped by size (A, B, C, etc.), and then the probable homologues are paired. A normal woman has two X chromosomes and a normal man, shown here, an X and a Y.*

How know which chromosome is which?



Down's Syndrome



(b)

(a)

19–4 (a) *Although children with Down's syndrome share certain physical characteristics, there is a wide range of mental capacity among these individuals.* (b) *The karyotype of a male with Down's syndrome caused by nondisjunction. Note that there are three chromosomes 21.*

Mechanism causing abnormality?

CHROMOSOMAL ABNORMALITIES CAUSED BY ERRORS in EGG + SPERM FORMATION (MEIOSIS)

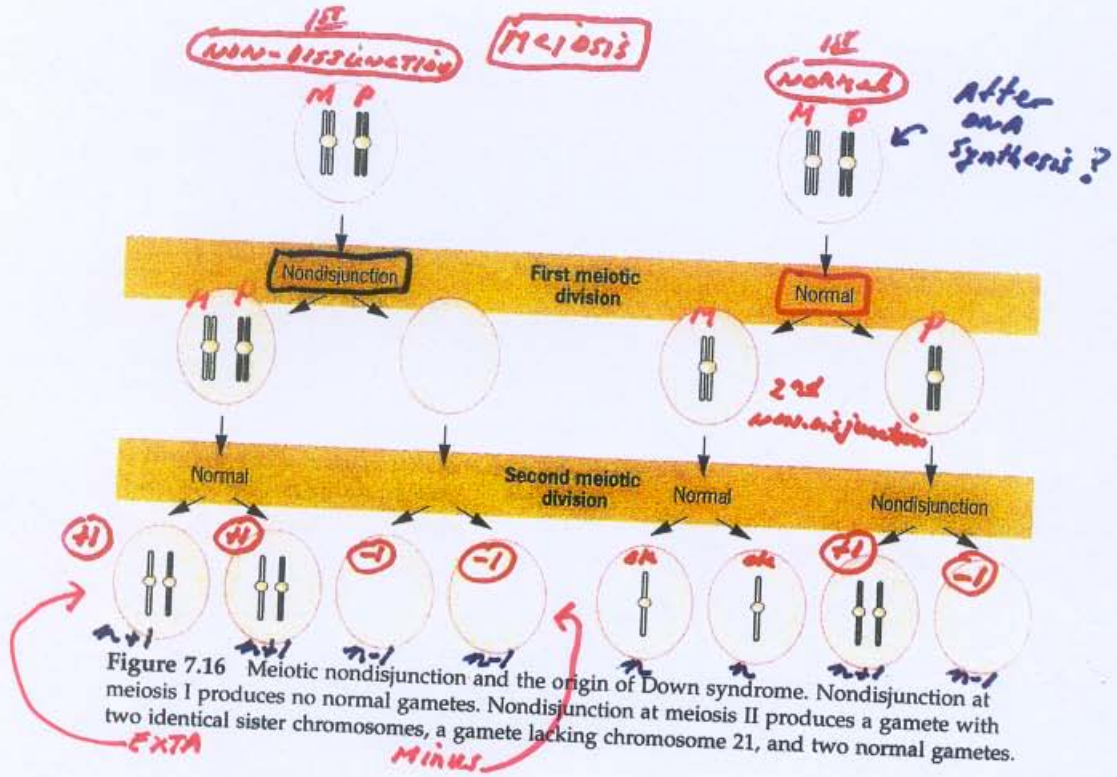


Figure 7.16 Meiotic nondisjunction and the origin of Down syndrome. Nondisjunction at meiosis I produces no normal gametes. Nondisjunction at meiosis II produces a gamete with two identical sister chromosomes, a gamete lacking chromosome 21, and two normal gametes.

ONLY PROBES OR BANDING PATTERN IDENTIFIES CHROMOSOME

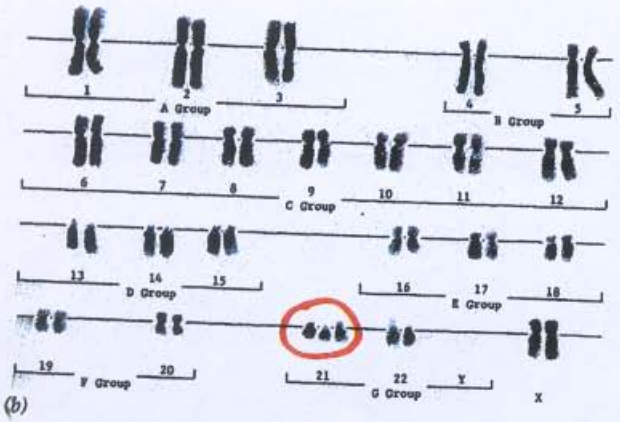


Figure 7.15 Down syndrome. (*a*) Facial features of a child with Down syndrome. (*b*) Karyotype of a child with Down syndrome, showing trisomy for chromosome 21 (47,XX, +21).

Detection of Extra Chromosome By In Situ Hybridization

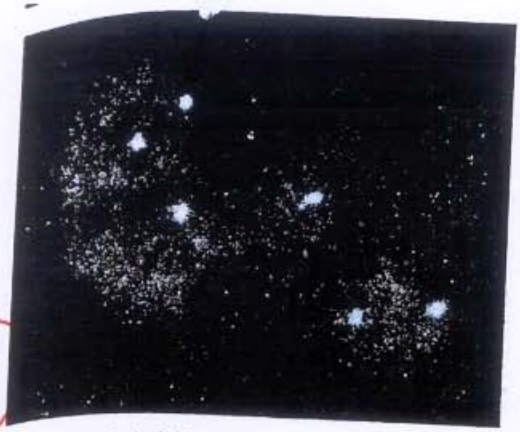


Fig. 3.13. Amniotic fluid cell nuclei of a fetus with trisomy 18 after CISS hybridization with the biotinylated Alu-PCR amplified YAC clone HTY 3045 (mapped to 18q23) detected with avidin-FITC. Nuclei were counterstained with propidium iodide

Note

3 chromosome 18's

Use of a Chromosome 18 Specific DNA Sequence

Amniotic cells

Recounting a genetic story

Roger H. Reeves

The DNA sequence of human chromosome 21, now published, provides indications that the total number of human genes has been overestimated, and is a valuable resource for research into Down syndrome.

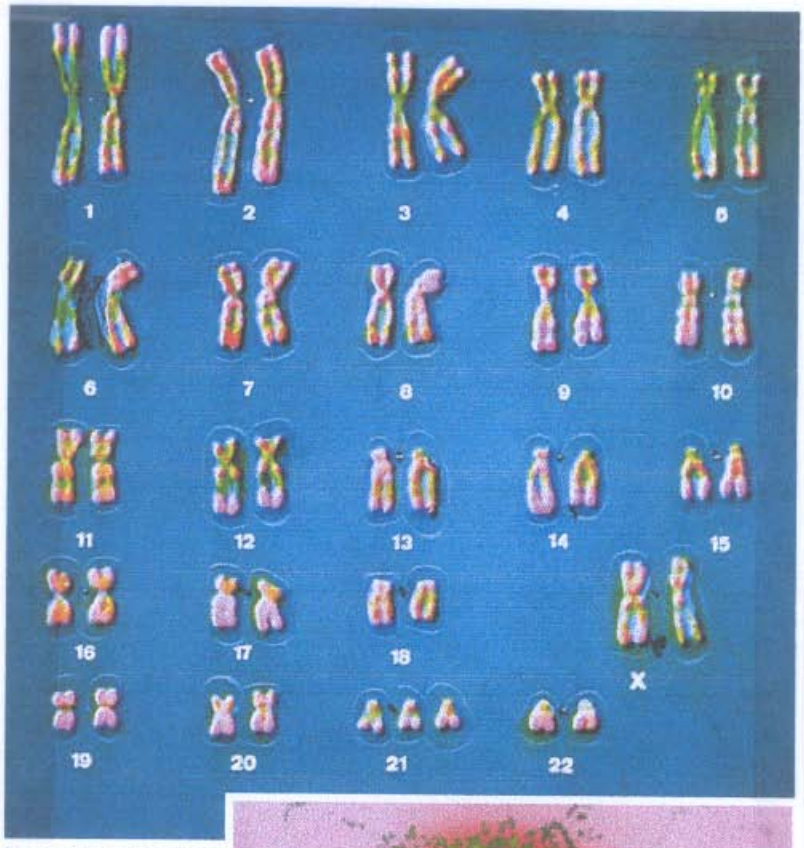


Figure 1 Chromosome 21 in context. Top, triplication of chromosome 21 is the genetic defect underlying Down syndrome. Bottom, transmission electron micrograph of chromosome 21, showing the long and short arms.

The DNA sequence of human chromosome 21

The chromosome 21 mapping and sequencing consortium

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Chromosome 21 is the smallest human autosome. An extra copy of chromosome 21 causes Down syndrome, the most frequent genetic cause of significant mental retardation, which affects up to 1 in 700 live births. Several anonymous loci for monogenic disorders and predispositions for common complex disorders have also been mapped to this chromosome, and loss of heterozygosity has been observed in regions associated with solid tumours. Here we report the sequence and gene catalogue of the long arm of chromosome 21. We have sequenced 33,546,361 base pairs (bp) of DNA with very high accuracy, the largest contig being 25,491,867 bp. Only three small clone gaps and seven sequencing gaps remain, comprising about 100 kilobases. Thus, we achieved 99.7% coverage of 21q. We also sequenced 281,116 bp from the short arm. The structural features identified include duplications that are probably involved in chromosomal abnormalities and repeat structures in the telomeric and pericentromeric regions. Analysis of the chromosome revealed 127 known genes, 98 predicted genes and 59 pseudogenes.

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A gene expression map of human chromosome 21 orthologues in the mouse

The HSA21 expression map initiative
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Ariel Ruiz i Altaba†
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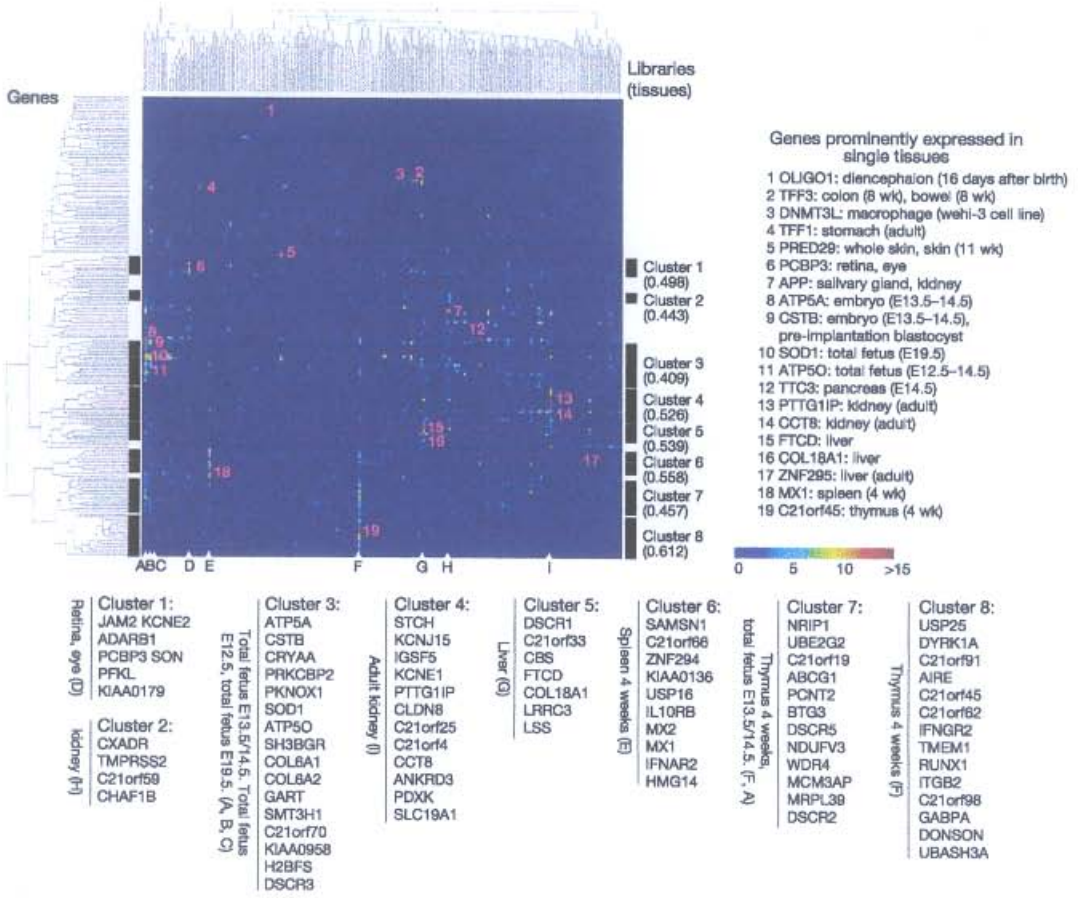


Figure 4 EST analysis. Matrix displaying the expression profiles of 159 mmu21 genes (rows) within 190 cDNA libraries (columns). Dendrograms used to reorder libraries (top) and genes (left) are shown, together with eight significant gene clusters (solid bars, left and right of the matrix together with correlation coefficients). The cluster composition is shown with corresponding libraries (A-I and indicated by white arrows at the bottom of the matrix). Coloured dots represent the number of ESTs found in a given library for each gene (see scale). Numbers in red refer to genes prominently expressed in single tissues, listed at the right. Interactive figure and details are given in the Supplementary Information. Gene symbols of human orthologues are used.

Chromosome 21

(1) ~ 200-300 genes

(2) Down's Syndrome Results From overexpression of these genes

(3) SOD / Superoxide Dismutase Gene

SOD converts oxygen radicals to H_2O_2
if SOD activity increase above enzyme activity to remove H_2O_2 (peroxidases) – peroxidase damage can occur to brain cells

(4) Amyloid Protein Gene

Amyloid plagues in brain → Down's individuals + Alzheimer individuals

(5) Ts65Dn mouse – TRisomy 16 – Genes similar to those on Human 21 – ∴ Model to understand Molecular Basis of Down's Syndrome.

Major chromosomal Defects in Humans

TABLE 7.1
Aneuploidy Resulting from Nondisjunction in Human Beings

Karyotype	*Chromosome Formula*	*Clinical Syndrome*	*Estimated Frequency at Birth*	*Phenotype*
47,+21	2n+1	Down	1/700	Short, broad hands with palmar crease, short stature, hyperflexibility of joints, mental retardation, broad head with round face, open mouth with large tongue, epicanthal fold.
47,+13	2n+1	Patau	1/20,000	Mental deficiency and deafness, minor muscle seizures, cleft lip and/or palate, cardiac anomalies, posterior heel prominence.
47,+18	2n+1	Edward	1/8000	Congenital malformation of many organs, low-set, malformed ears, receding mandible, small mouth and nose with general elfin appearance, mental deficiency, horseshoe or double kidney, short sternum, 90 percent die within first six months after birth.
SEX CHROMOSOMES				
−1 45,X	2n−1	Turner	1/2500 female births	Female with retarded sexual development, usually sterile, short stature, webbing of skin in neck region, cardiovascular abnormalities, hearing impairment.
+1 47,XXY	2n+1	Klinefelter	1/500 male births	Male, subfertile with small testes, developed breasts, feminine-pitched voice, knock knees, long limbs.
+2 48,XXXY	2n+2			
+2 48,XXYY	2n+2			
+3 49,XXXXY	2n+3			
+4 50,XXXXXY	2n+4			
47,XXX	2n+1	Triplo-X	1/700	Female with usually normal genitalia and limited fertility, slight mental retardation.

① The XYY Story — Science goes wrong!
② How obtain an XX male?

Most Changes in Chromosome Number Are Lethal

Table 9.2 Chromosome abnormalities per 100,000 recognized human pregnancies

1/7 or 15%!

Live Births

	15,000 spontaneous abortions	85,000 live births
Trisomy		
A: 1	0	0
A: 2	159	0
A: 3	53	0
B: 4	95	0
B: 5	0	0
C: 6–12	561	0
D: 13	128	17
D: 14	275	0
D: 15	318	0
E: 16	1229	0
E: 17	10	0
E: 18	223	13
F: 19–20	52	0
G: 21	350	113
G: 22	424	0
Sex chromosomes		
XYY	4	46
XXY	4	44
XO	1350	8
XXX	21	44
Translocations		
Balanced	14	164
Unbalanced	225	52
Polyploid		
Triploid	1275	0
Tetraploid	450	0
Other (mosaics, etc.)	280	49
Total	7500	550

Hypothesis?

Lead to Spontaneous Abortion

Explain why 1 Xtra chromosome can lead to Abortion?

Explain How Chromosomes 13, 18, & 21 can lead to live births with severe abnormalities & other chromosomes ↳ death

How obtain polyploid fetus?

(1) 15% of conceptions lead to Spontaneous Abortions

(2) Half of these are due to chromosome Abnormalities

(3) ~ 0.65% of Live Birth mutations due to chromosome Abnormalities

(4) ~ 1.2% of Live Birth Mutations due to DNA Changes / Point Mutations

~ 2% of Live Births Have Genetic Defects That are Visible

550/85000 = 0.65%

The other 7500 are caused by Mutations!

1/50!

FREQUENCY OF Gene and Chromosomal Mutations in Live Births

Live Births

Table 9-1. Relative Incidence of Human Ill Health due to Gene Mutation and to Chromosome Mutation

Live Births

Type of mutation	Percentage of live births
Gene mutation (Gene) GENE	
Autosomal dominant	0.90
Autosomal recessive	0.25
X-linked	0.05
Total gene mutation (1.2%)	1.20
Chromosome mutation (Chromosomal) CHROMOSOMAL	
Autosomal trisomies (mainly Down syndrome)	0.14
Other unbalanced autosomal aberrations	0.06
Balanced autosomal aberrations	0.19
Sex chromosomes	
XYY, XXY, and other ♂♂	0.17
XO, XXX, and ♀♀	0.05
Total chromosome mutation (0.61%)	0.61

total 1.81%

GENE MUTATION →

CHROMOSOME ABNORMALITIES →

1.2% of Live Births = Mutations

0.61% of Live Births = Chromosome defects

~2% of all Live Births have Genetic defects

Note: 15% of conceptions lead to Spontaneous Abortions. only 85% give rise to live births

HOW RELATE TO DNA TESTING – ELIMINATE Lottery?

A Large # of Spontaneous Abortions Caused by Chromosome & DNA Changes. Mutations also Affect Large #'s of Children who are Born

SPERM & EGG FORMATION

♂ ♀

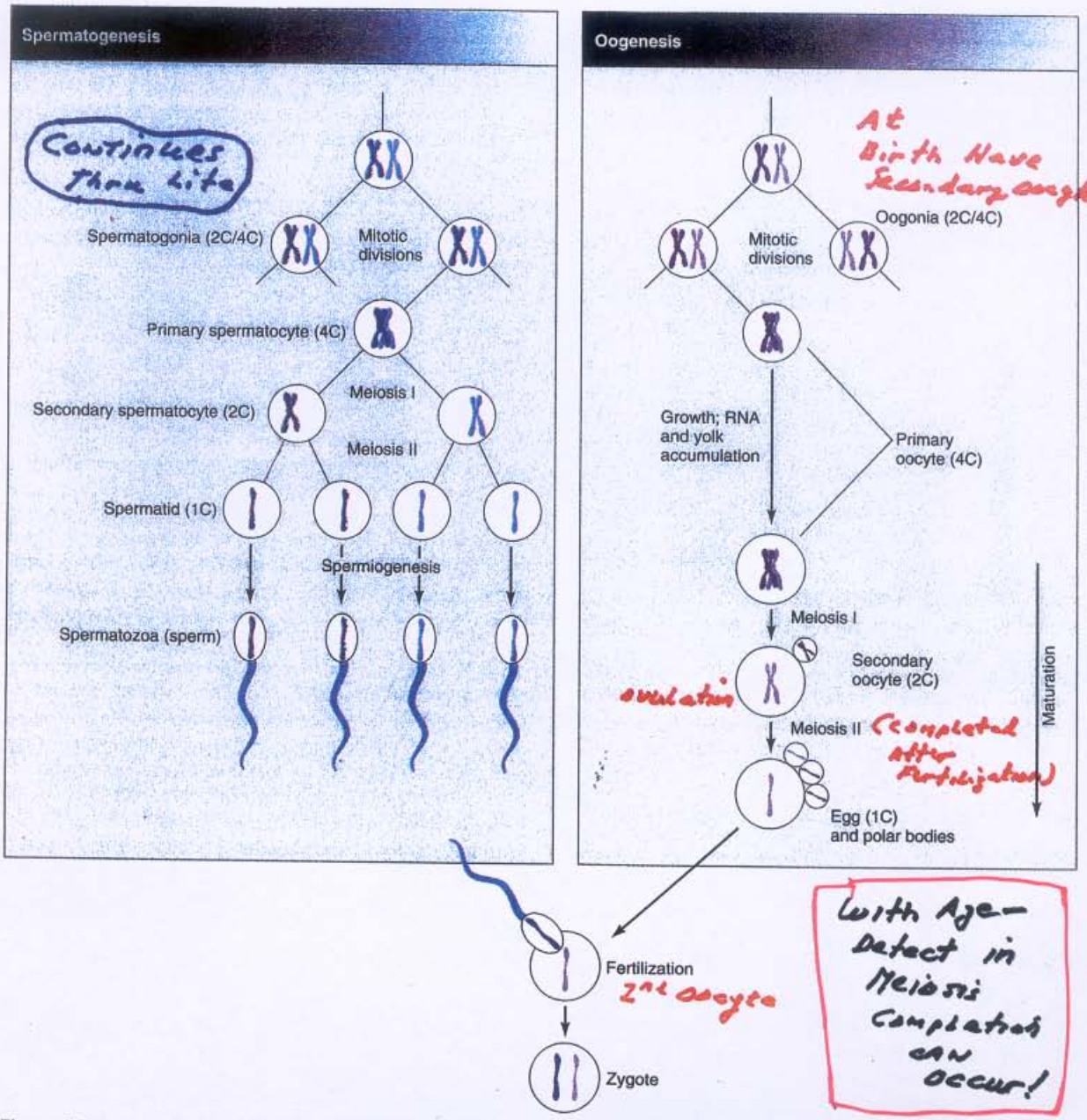
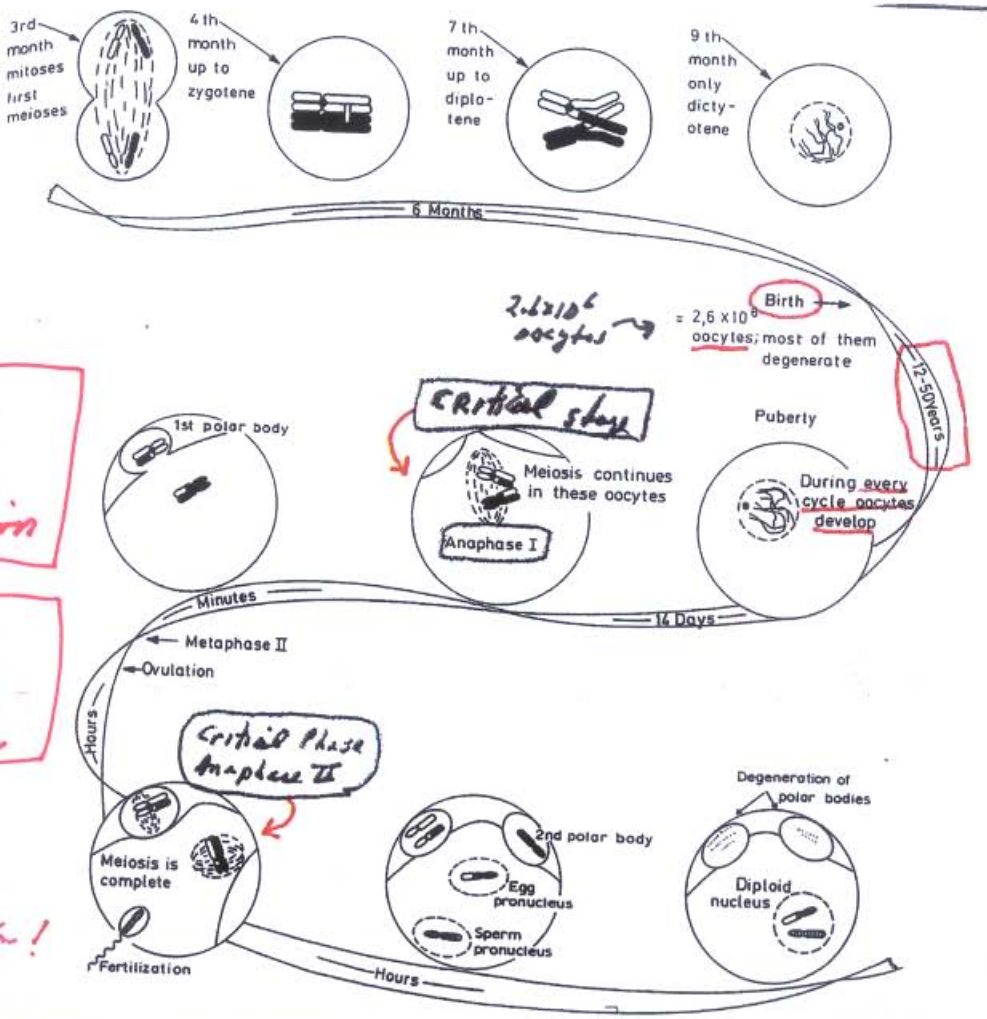


Figure 3.5 Comparison of spermatogenesis and oogenesis. Primordial germ cells divide mitotically, producing spermatogonia in males and oogonia in females. These cells are diploid, containing two or four genomic complements (2C or 4C), depending on their stage in the mitotic cycle. Before the gonia enter meiosis, their DNA replicates. They are then called primary spermatocytes or oocytes. After the first meiotic division, they contain two genomes (2C) and are called secondary spermatocytes or oocytes. After the second meiotic division, they are haploid (1C) spermatids or eggs. Note that the two rounds of meiosis produce four haploid spermatids, each of which develops into a spermatozoon, but only one egg. The egg's three small sister cells, known as polar bodies, have no known function and degenerate. Often the first polar body does not divide, so that only a total of two polar bodies is formed. Depending upon the species, eggs are fertilized at various stages of meiosis (see Fig. 3.18).

DEFECTS AGE RELATED — ♀ AND ♂

Meiosis in Egg Formation is Completed after Fertilization



Meiosis I PRIOR to Ovulation

MEIOSIS II After Fertilization

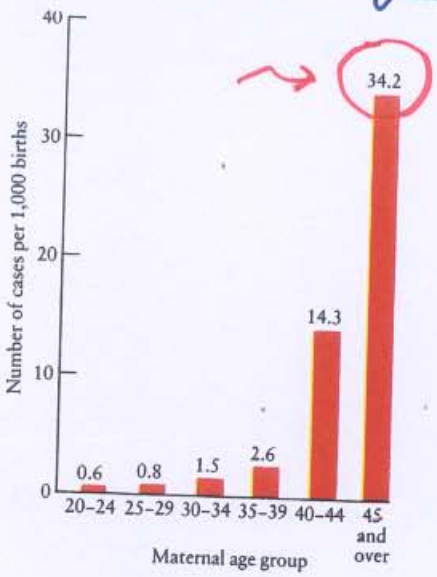
Egg forms after fertilization!

Fig. 2.21. Meiosis in the human female. Meiosis starts after 3 months of development. During childhood the cytoplasm of oocytes increases in volume, but the nucleus remains unchanged. About 90% of all oocytes degenerate at the onset of puberty. During the first half of every month the luteinizing hormone *(LH)* of the pituitary stimulates meiosis which is now almost completed (end of the prophase that began during embryonic age; metaphase I, anaphase I, telophase I and – within a few minutes – prophase II and metaphase II). Then meiosis stops again. A few hours after metaphase I is reached ovulation is induced by *LH*. Fertilization occurs in the fallopian tube. Then the second meiotic division is completed. Nuclear membranes are formed around the maternal and paternal chromosomes. After some hours the two "pronuclei" fuse, and the first cleavage division begins. (From Bresch and Hausmann 1972)

And all "Eggs" are Present at Birth

∴ Meiotic Errors increase with Age!

FREQUENCIES OF CHILDREN BORN WITH CHROMOSONAL DEFECTS INCREASES WITH AGE OF MOTHER



19–6 *The frequencies of births of infants with Down's syndrome in relation to the ages of the mothers. The number of cases shown for each age group represents the occurrence of Down's syndrome in every 1,000 live births by mothers in that group. As you can see, the risk of having a child with Down's syndrome increases rapidly after the mother's age exceeds 40. An increased risk is also thought to occur after the father's age exceeds 55.*

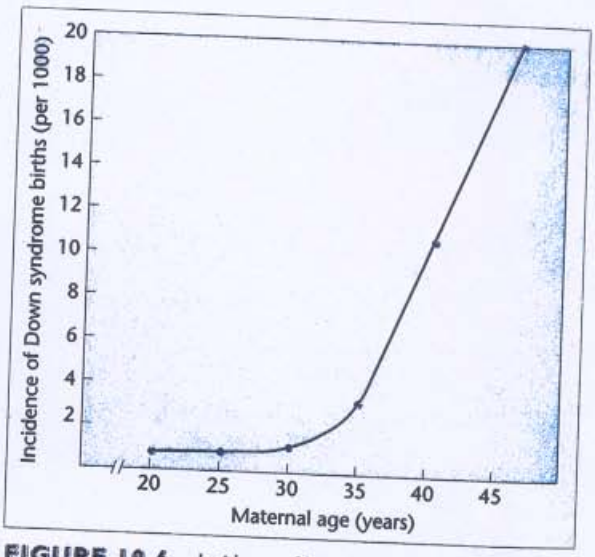
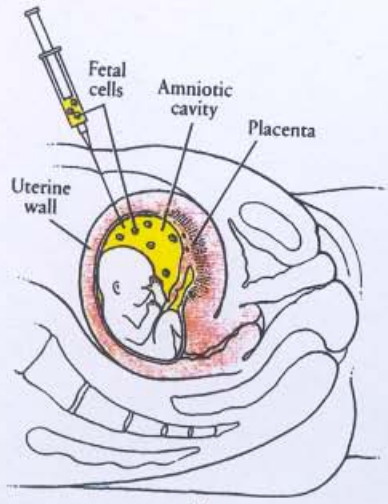


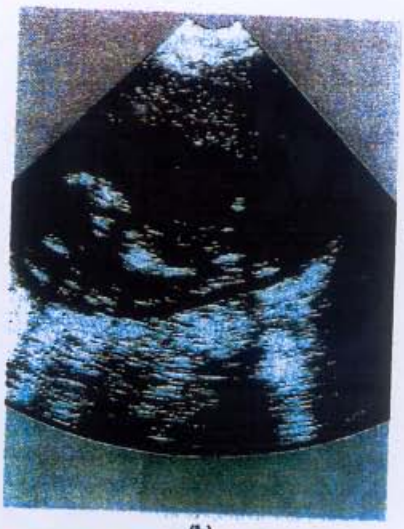
FIGURE 10.6 Incidence of Down syndrome births contrasted with maternal age.

nificantly older and arrested longer than those they ovulated 10 or 20 years previously. However, it is not yet known whether ovum age is the cause of the increased incidence of nondisjunction leading to Down syndrome.

These statistics are the basis of a serious issue facing parents when pregnancy occurs late in a woman's reproductive years. Genetic counseling early in such pregnan-



(a)



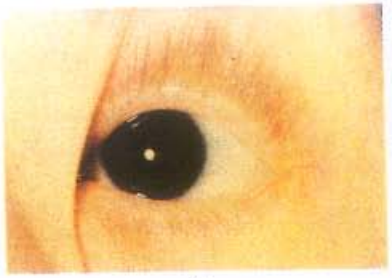
(b)

IMPORTANCE OF PRENATAL CHROMOSOME TESTING

Large deletions also cause Genetic Abnormalities

19–7 (a) *A chromosomal abnormality associated with cancer. The chromosomes shown here have been stained to reveal banding patterns. The chromosome on the left is normal. The one on the right has a deletion, shown by the smaller size of the bracket. Such deletions have been found in children with Wilms' tumor.* (b) *The left eye of a 15-year-old boy who has this chromosomal deletion and who developed Wilms' tumor in infancy. Note the absence of an iris. An older half-brother and a maternal aunt also had aniridia and developed Wilms' tumor at an early age. Another brother and the boy's mother are phenotypically normal. Analysis of the mother's chromosomes revealed that although she carries the deletion in chromosome 11, the missing segment is present in her cells in chromosome 2. Almost all other chromosomal abnormalities associated with cancer have occurred only in somatic cells and are not inherited.*

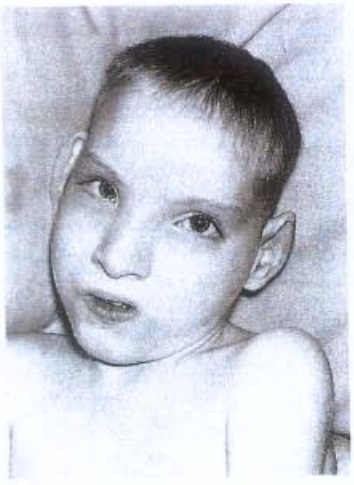
(a)



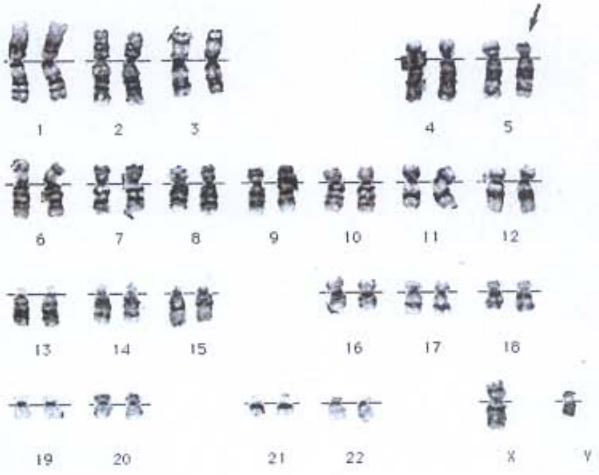
(b)

were used to correlate Gene with Chromosome Region

How correlate gene with Locus?



(a)



(b)

Figure 7.18 *Cri-du-chat* syndrome. (*a*) Patient with *cri-du-chat* syndrome. (*b*) Karyotype of infant with *cri-du-chat* syndrome, 46, XY(5p-). There is a deletion in the short arm of chromosome 5 (arrow).

Rearranged Chromosomes ALSO Lead to Genetic Abnormalities

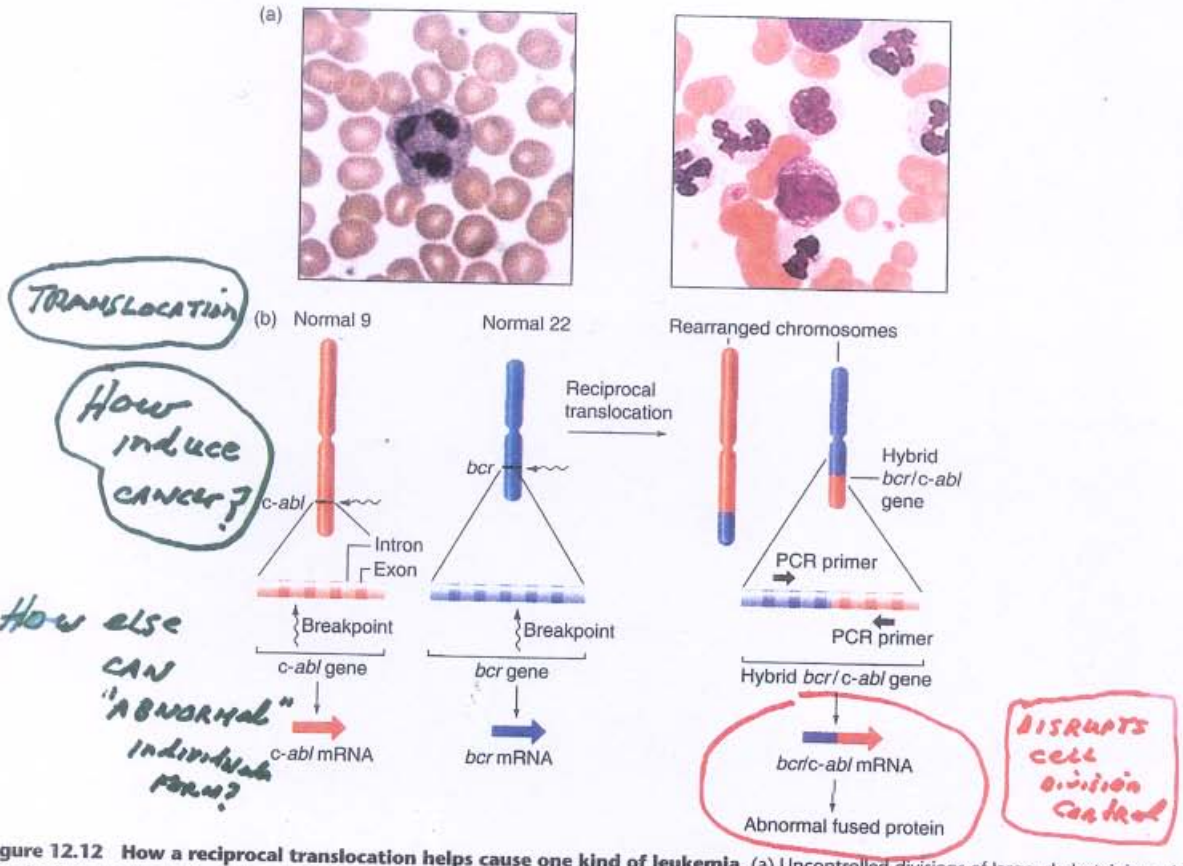
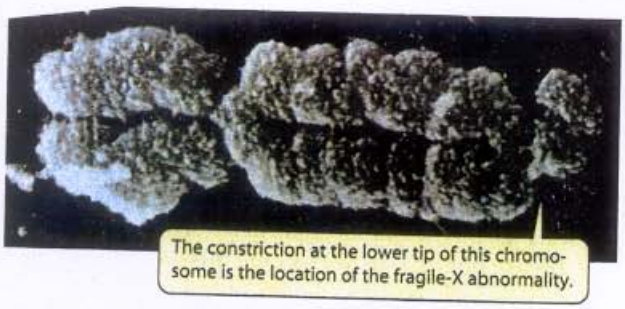


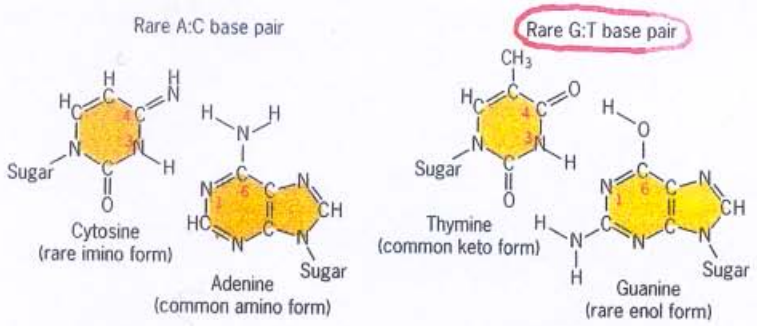
Figure 12.12 How a reciprocal translocation helps cause one kind of leukemia. (a) Uncontrolled divisions of large, dark-staining white blood cells in the blood of a leukemia patient (right) produce a higher than normal ratio of white to red blood cells than that of a normal individual (left). (b) A reciprocal translocation between chromosomes 9 and 22 contributes to chronic myelogenous leukemia. This rearrangement makes an abnormal hybrid gene composed of part of the c-*abl* gene on chromosome 9 and part of the *bcr* gene on chromosome 22. The hybrid gene produces a mRNA with sequences from both c-*abl* and *bcr*, and this hybrid mRNA is translated into an abnormal fused protein that disrupts controls on cell division. Black arrows indicate PCR primers that will generate a PCR product only in DNA containing the hybrid gene.



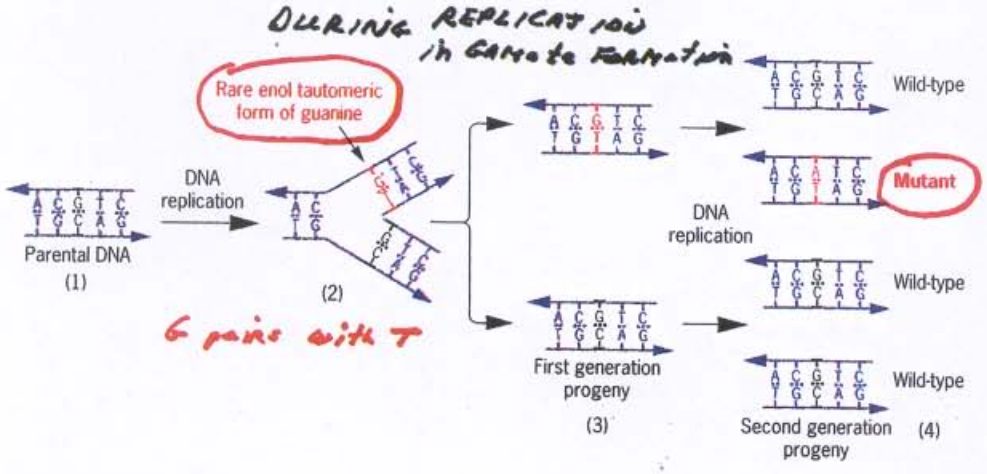
Chromosome Breakage/Deletion

18.5 A Fragile-X Chromosome at Metaphase
The chromosomal abnormality that causes the mental retardation symptomatic of fragile-X syndrome shows up physically as a constriction.

MANY CHANGES in DNA Sequence ALSO Occur



(a) Hydrogen-bonded A:C and G:T base pairs that form when cytosine and guanine are in their rare imino and enol tautomeric forms.



(b) Mechanism by which tautomeric shifts in the bases in DNA cause mutations.

Figure 14.14 The effects of tautomeric shifts in the nucleotides in DNA on (*a*) base-pairing and (*b*) mutation. Rare A:C and G:T base pairs like those shown in (*a*) also form when thymine and adenine are in their rare enol and imino forms, respectively. (*b*) A guanine (1) undergoes a tautomeric shift to its rare enol form (G*) at the time of replication (2). In its enol form, guanine pairs with thymine (2). During the subsequent replication (3 to 4), the guanine shifts back to its more stable keto form. The thymine incorporated opposite the enol form of guanine (2) directs the incorporation of adenine during the next replication (3 to 4). The net result is a G:C to A:T base-pair substitution.

1.2% of Live Births Affected by these Mutations

MOST CHANGES IN GENOME IN NON-CODING DNA & DO NOT LEAD TO MUTATIONS

RECALL - ONLY 1% of GENOME = EXONS!

(84)

SINGLE BASE PAIR CHANGES (SNPs) ARE FREQUENT IN GENOME

TABLE 9.1 Five Classes of DNA Polymorphism

Class	Cause	Rate of Mutation per Locus per Gamete	Frequency in Genome	Number per Human Genome (on average)
Single base	Mutagens or replication errors	10^{-8}–10^{-9}	1/700 bp	3 million
Microsatellite	Slippage during replication	10^{-3}	1/30,000 bp	100,000
Minisatellite	Unequal crossovers	10^{-3}	Unknown; discovered by chance	Fewer than 100 families known, yielding 1000 copies in all
Deletions	Mutagens; unequal crossovers	Extremely rare	Very low	0 – a few
Duplications	Mutagens; unequal crossovers	Extremely rare	Very low	0 – a few
Other insertions (excluding those resulting from micro- or minisatellite recombination)	Transposable elements	Extremely rare	Very low	0 – a few
Complex haplotype (any locus of 5 kb or more)	Any of the above	Combination of the above	Not applicable	Not applicable

DETECTED USING RESTRICTION ENZYMES (The OLD FASHIONED WAY) OR BY DIRECT DNA sequencing of 2 individuals' Genes / Genomes

ONLY MOLECULAR APPROACHES CAN DETECT SNPs

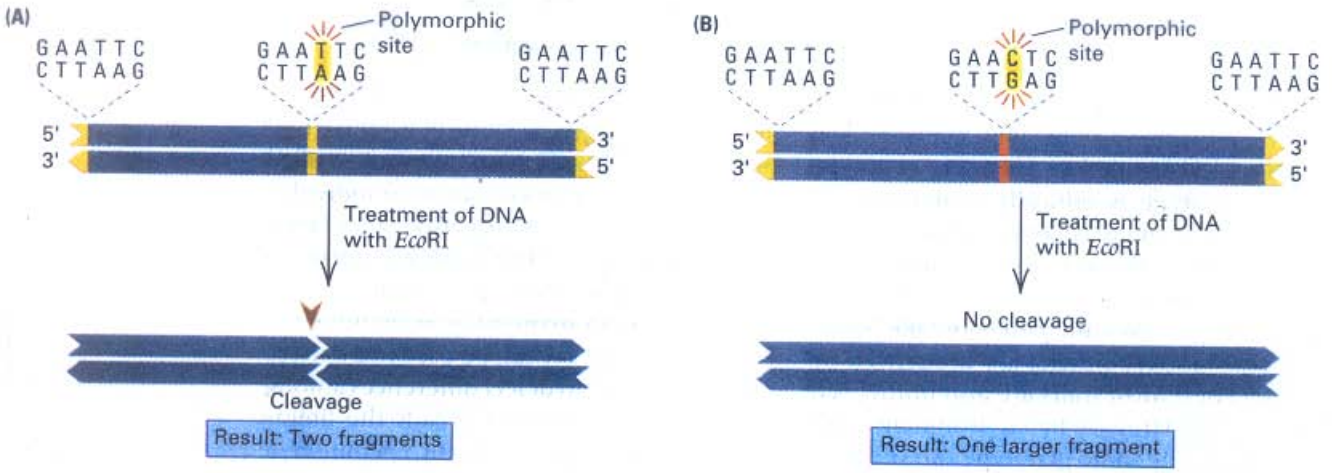
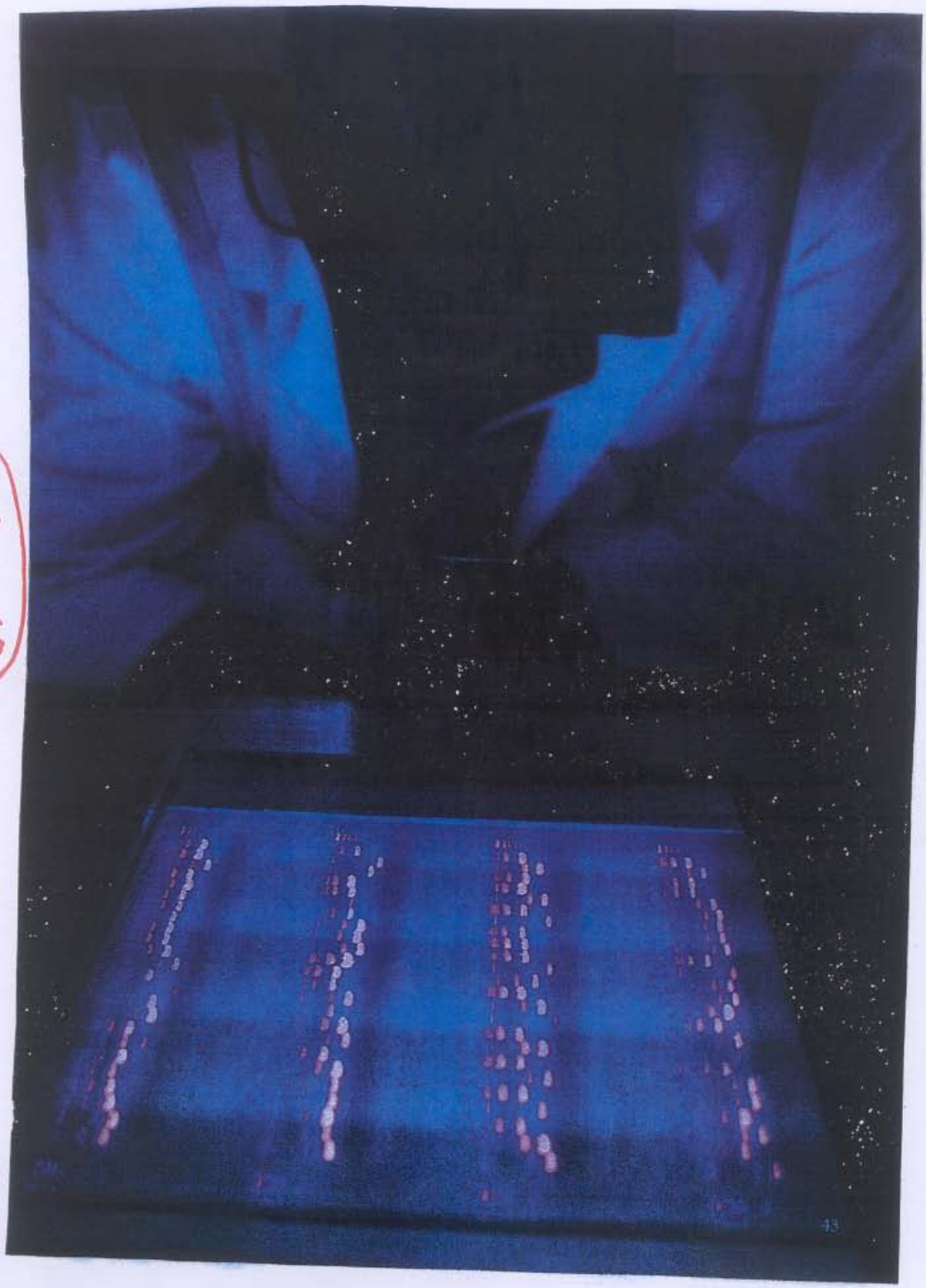


Figure 2.23 A minor difference in the DNA sequence of two molecules can be detected if the difference eliminates a restriction site. (A) This molecule contains three restriction sites for *Eco*RI, including one at each end. It is cleaved into two fragments by the enzyme. (B) This molecule has an altered *Eco*RI site in the middle, in which 5'-GAATTC-3' becomes 5'-GAACTC-3'. The altered site cannot be cleaved by *Eco*RI, so treatment of this molecule with *Eco*RI results in one larger fragment.

The old fashioned way to detect RFLPs —

DNA Blot
PCR
ASO

Visualization of Polymorphisms Due to Mutations



RFLP due to point mutation

NOT VNTR

OR By Sequencing . . . . .

THE CURRENT METHOD

A 2.91-billion base pair (bp) consensus sequence of the euchromatic portion of the human genome was generated by the whole-genome shotgun sequencing method. The 14.8-billion bp DNA sequence was generated over 9 months from 27,271,853 high-quality sequence reads (5.11-fold coverage of the genome) from both ends of plasmid clones made from the DNA of five individuals. Two assembly strategies—a whole-genome assembly and a regional chromosome assembly—were used, each combining sequence data from Celera and the publicly funded genome effort. The public data were shredded into 550-bp segments to create a 2.9-fold coverage of those genome regions that had been sequenced, without including biases inherent in the cloning and assembly procedure used by the publicly funded group. This brought the effective coverage in the assemblies to eightfold, reducing the number and size of gaps in the final assembly over what would be obtained with 5.11-fold coverage. The two assembly strategies yielded very similar results that largely agree with independent mapping data. The assemblies effectively cover the euchromatic regions of the human chromosomes. More than 90% of the genome is in scaffold assemblies of 100,000 bp or more, and 25% of the genome is in scaffolds of 10 million bp or larger. Analysis of the genome sequence revealed 26,588 protein-encoding transcripts for which there was strong corroborating evidence and an additional ~12,000 computationally derived genes with mouse matches or other weak supporting evidence. Although gene-dense clusters are obvious, almost half the genes are dispersed in low G+C sequence separated by large tracts of apparently noncoding sequence. Only 1.1% of the genome is spanned by exons, whereas 24% is in introns, with 75% of the genome being intergenic DNA. Duplications of segmental blocks, ranging in size up to chromosomal lengths, are abundant throughout the genome and reveal a complex evolutionary history. Comparative genomic analysis indicates vertebrate expansions of genes associated with neuronal function, with tissue-specific developmental regulation, and with the hemostasis and immune systems. DNA sequence comparisons between the consensus sequence and publicly funded genome data provided locations of 2.1 million single-nucleotide polymorphisms (SNPs). A random pair of human haploid genomes differed at a rate of 1 bp per 1250 on average, but there was marked heterogeneity in the level of polymorphism across the genome. Less than 1% of all SNPs resulted in variation in proteins, but the task of determining which SNPs have functional consequences remains an open challenge.

1 bp change per 1250 bp on average between 2 genomes

21% cause protein change - why?

SNPs

Single Nucleotide Polymorphisms

MARKERS !!

SNPs Are MARKERS

- use SNPs to determine Linkage with disease Genes - Markers
- Associate with adverse drug reactions
- Associate with predisposition to heart disease, etc.

PEOPLE, GROUPS, DISEASES, ORIGINS

Nature 409, 928-933 (2001)

A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms

The International SNP Map Working Group*

* *A full list of authors appears at the end of this paper.*

5.8 x 10^6 now 2005!

We describe a map of 1.42 million single nucleotide polymorphisms (SNPs) distributed throughout the human genome, providing an average density on available sequence of one SNP every 1.9 kilobases. These SNPs were primarily discovered by two projects: The SNP Consortium and the analysis of clone overlaps by the International Human Genome Sequencing Consortium. The map integrates all publicly available SNPs with described genes and other genomic features. We estimate that 60,000 SNPs fall within exon (coding and untranslated regions), and 85% of exons are within 5 kb of the nearest SNP. Nucleotide diversity varies greatly across the genome, in a manner broadly consistent with a standard population genetic model of human history. This high-density SNP map provides a public resource for defining haplotype variation across the genome, and should help to identify biomedically important genes for diagnosis and therapy.

Inherited differences in DNA sequence contribute to phenotypic variation, influencing an individual's anthropometric characteristics, risk of disease and response to the environment. A central goal of genetics is to pinpoint the DNA variants that contribute most significantly to population variation in each trait. Genome-wide linkage analysis and positional cloning have identified hundreds of genes for human diseases[1] (http://ncbi.nlm. nih.gov/OMIM), but nearly all are rare conditions in which mutation of a single gene is necessary and sufficient to cause disease. For common diseases, genome-wide linkage studies have had limited success, consistent with a more complex genetic architecture. If each locus contributes modestly to disease aetiology, more powerful methods will be required.

One promising approach is systematically to explore the limited set of common gene variants for association with disease[2–4]. In the human population most variant sites are rare, but the small number of common polymorphisms explain the bulk of heterozygosity[3] (see also refs 5–11). Moreover, human genetic diversity appears to be limited not only at the level of individual polymorphisms, but also in the specific combinations of alleles (haplotypes) observed at closely linked sites[8,11–14]. As these common variants are responsible for most heterozygosity in the population, it will be important to assess their potential impact on phenotypic trait variation.

If limited haplotype diversity is general, it should be practical to define common haplotypes using a dense set of polymorphic markers, and to evaluate each haplotype for association with disease. Such haplotype-based association studies offer a significant advantage: genomic regions can be tested for association without requiring the discovery of the functional variants. The required density of markers will depend on the complexity of the local haplotype structure, and the distance over which these haplotypes extend, neither of which is yet well defined.

Current estimates (refs 13–17) indicate that a very dense marker map (30,000–1,000,000 variants) would be required to perform haplotype-based association studies. Most human sequence variation is attributable to SNPs, with the rest attributable to insertions or deletions of one or more bases, repeat length polymorphisms and rearrangements. SNPs occur (on average) every 1,000–2,000 bases when two human chromosomes are compared[5,6,9,18–20], and are thus present at sufficient density for comprehensive haplotype analysis. SNPs are binary, and thus well suited to automated, high-throughput genotyping. Finally, in contrast to more mutable markers, such as microsatellites[21], SNPs have a low rate of recurrent mutation, making them stable indicators of human history. We have constructed a SNP map of the human genome with sufficient density to study human haplotype structure, enabling future study of human medical and population genetics.

Identification and characteristics of SNPs

The map contains all SNPs that were publicly available in November 2000. Over 95% were discovered by The SNP Consortium (TSC) and the public Human Genome Project (HGP). TSC contributed 1,023,950 candidate SNPs (http:// snp.cshl.org) identified by shotgun sequencing of genomic fragments drawn from a complete (45% of data) or reduced (55% of data) representation of the human genome[18,22]. Individual contributions were: Whitehead Institute, 589,209 SNPs from 2.57 million (M) passing reads; Sanger Centre, 262,279 SNPs from 1.16M passing reads; Washington University, 172,462 SNPs from 1.69M passing reads. TSC SNPs were discovered using a publicly available panel of 24 ethnically diverse individuals[23]. Reads were aligned to one another and to the available genome sequence, followed by detection of single base differences using one of two validated algorithms: Polybayes[24] and the neighbourhood quality standard (NQS[18,22]).

An additional 971,077 candidate SNPs were identified as sequence differences in regions of overlap between large-insert clones (bacterial artificial chromosomes (BACs) or P1-derived artificial chromosomes (PACs)) sequenced by the HGP. Two groups (NCBI/Washington University (556,694 SNPs): G.B., P.Y.K. and S.S.; and The Sanger Centre (630,147SNPs): J.C.M. and D.R.B.) independently analysed these overlaps using the two detection algorithms. This approach contributes dense clusters of SNPs throughout the genome. The remaining 5% of SNPs were discovered in gene-based studies, either by automated detection of single base differences in clusters of overlapping expressed sequence tags[24–28] or by targeted resequencing efforts (see ftp://ncbi.nlm.nih.gov/snp/human/submit_format/*/*publicat.rep. gz).

It is critical that candidate SNPs have a high likelihood of representing true polymorphisms when examined in population studies. Although many methods and contributors are represented on the map (see above), most SNPs (> 95%) were contributed by two large-scale efforts that uniformly applied automated methods.

(1) did not know who (2) ALL gave consent + approved by IRB cannot use for group specific studies

P9

MOST SNPs ARE NOT IN CODING SEQUENCES & HAVE NO PHENOTYPIC EFFECTS!

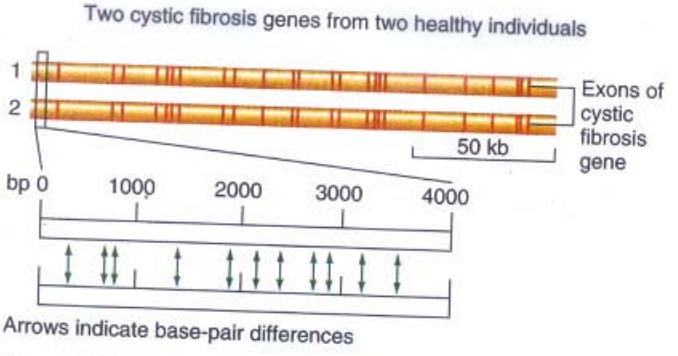


Figure 9.2 Base-pair differences between DNA cloned from the cystic fibrosis locus of two healthy individuals. These base-pair differences have no phenotypic effect; apparently they neither encode nor regulate expressed regions of the gene.

$\frac{3 \times 10^9 \text{ bp/genome}}{600 \text{ bp/SNP}} = 5 \times 10^6 \text{ SNPs/genome!}$

EACH OF US DIFFERS BY 5×10^9 bp !!!!!!!!

~ 1.5% of genome!

MOST OCCUR IN INTERGENIC / INTRON REGIONS & ARE USEFUL IN FORENSICS & DISEASE MARKING

USING SNPs as GENE MARKERS

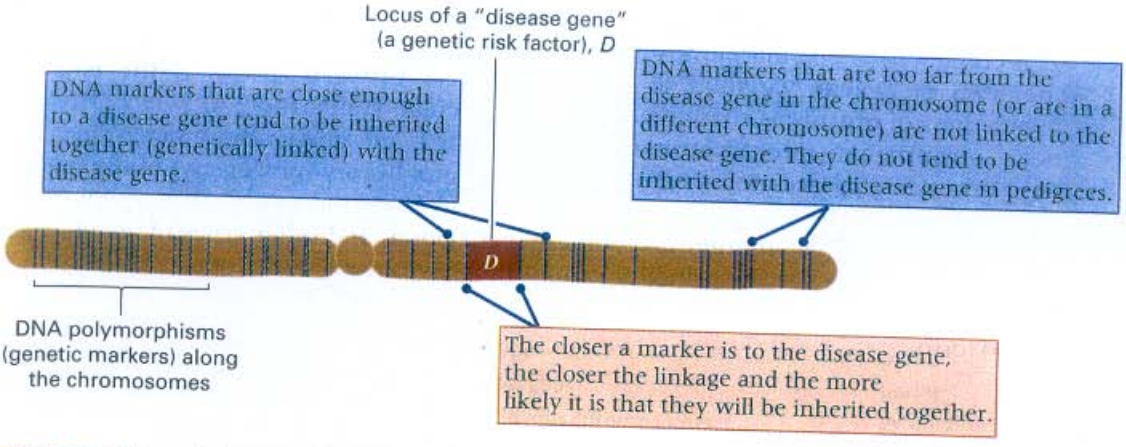


Figure 2.29 Concepts in genetic localization of genetic risk factors for disease. Polymorphic DNA markers (indicated by the vertical lines) that are close to a genetic risk factor (*D*) in the chromosome tend to be inherited together with the disease itself. The genomic location of the risk factor is determined by examining the known genomic locations of the DNA polymorphisms that are linked with it.

MAP GENES FOR DISEASE SUSCEPTIBILITY — GENES ENCODING COMPLEX (MULTI-genic) TRAITS (e.g., Heart Disease, Depression, DRUG Sensitivity, Obesity)

SNPs = INDIVIDUAL GENE PROFILE

↳ INDIVIDUAL Medicine!