ASOs or Allele Specific Oligonucleotide Probes can be used to detect Specific Alleles/RFLPs/SNPs

This is the fastest/simplest Approach to Fingerprinting or Monitoring disease loci - it utilizes PCR ⊕ Specific Annealing Conditions!

AT HIGH TEMPERATURE ONLY A PERFECT MATCH CAN ANNEAL SUCCESFULLY! ONE MISMATCHED BASE PREVENTS HYBRID FORMATION!!!

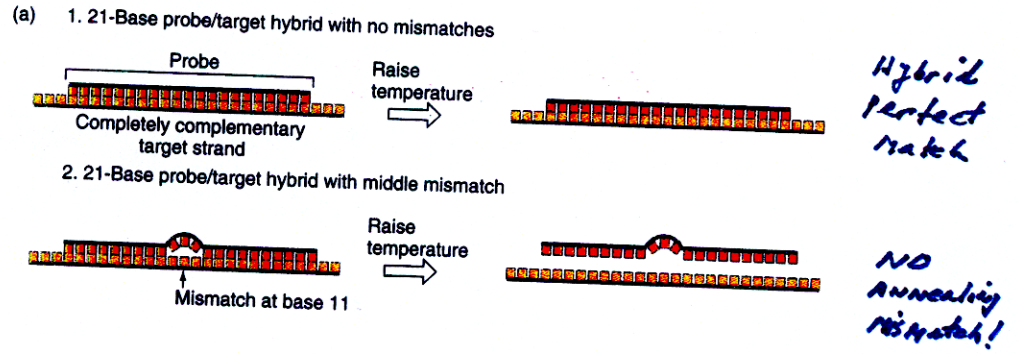


Figure 9.8 Short hybridization probes can distinguish single-base mismatches, longer probes cannot. (a) Researchers allow hybridization to occur between a short 21-base probe and two different target sequences. (1) A perfect match between probe and target extends across all 21 bases. When the temperature rises, this hybrid has enough hydrogen bonds to remain intact. (2) With a single-base mismatch in the middle of the probe, the effective length of the probe-target hybrid is only 10 bases. When the temperature rises, this hybrid does not have enough hydrogen bonds to remain intact, and it falls apart.

A Simpler More Inexpensive Way to Find SNPs/RFLPs!

Using ASOs + BABI test to detect Mutant Cystic fibrosis Alleles in Post-fertilization Embryos

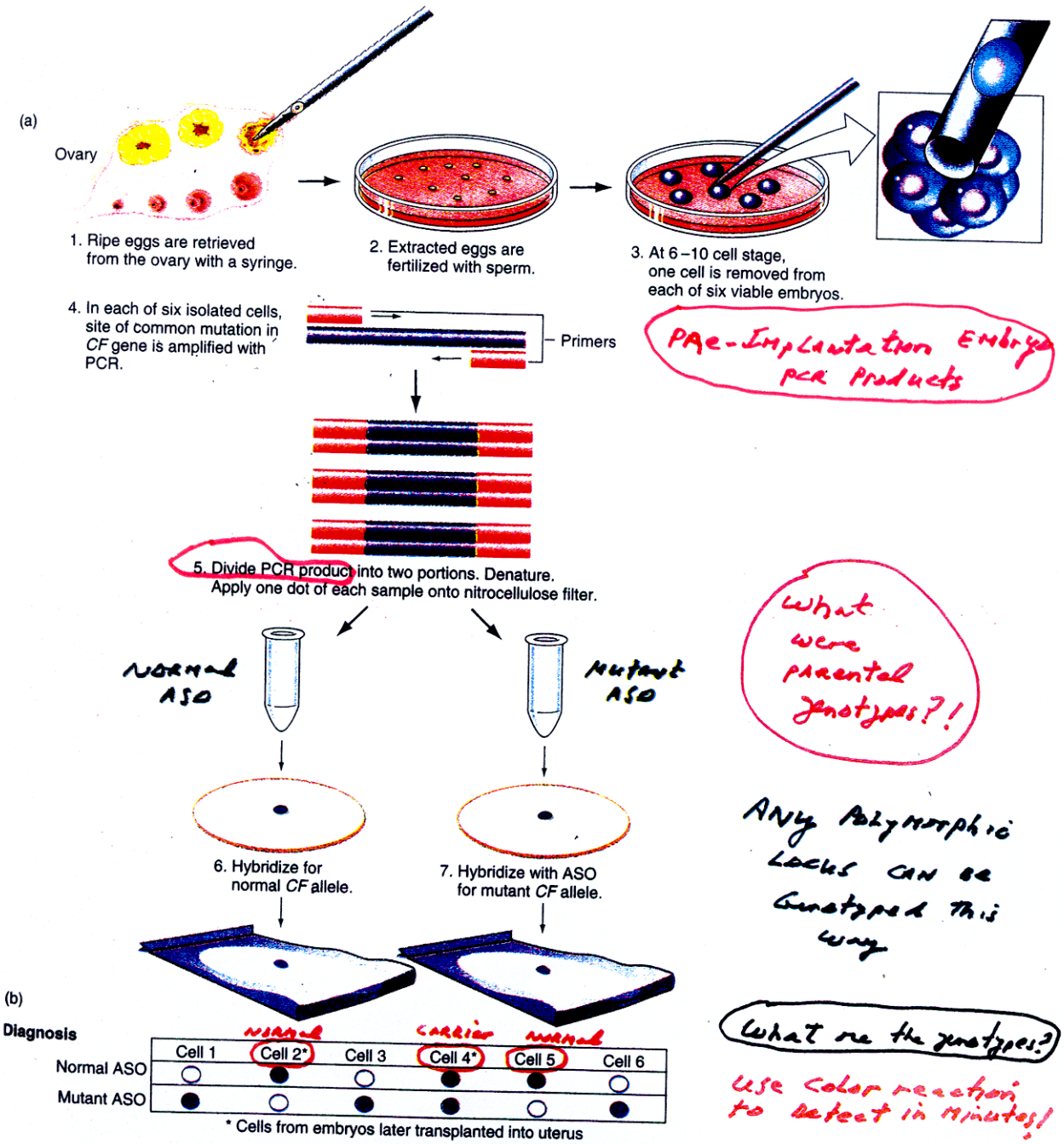


Figure 9.1 Detecting the cystic fibrosis genotype of embryonic cells. (a) *In vitro* fertilization and preimplantation diagnosis. (b) Cell 2 is homozygous for the normal allele; cell 4 is heterozygous for the *CF* mutation.

USE OF ASOs in Forensics

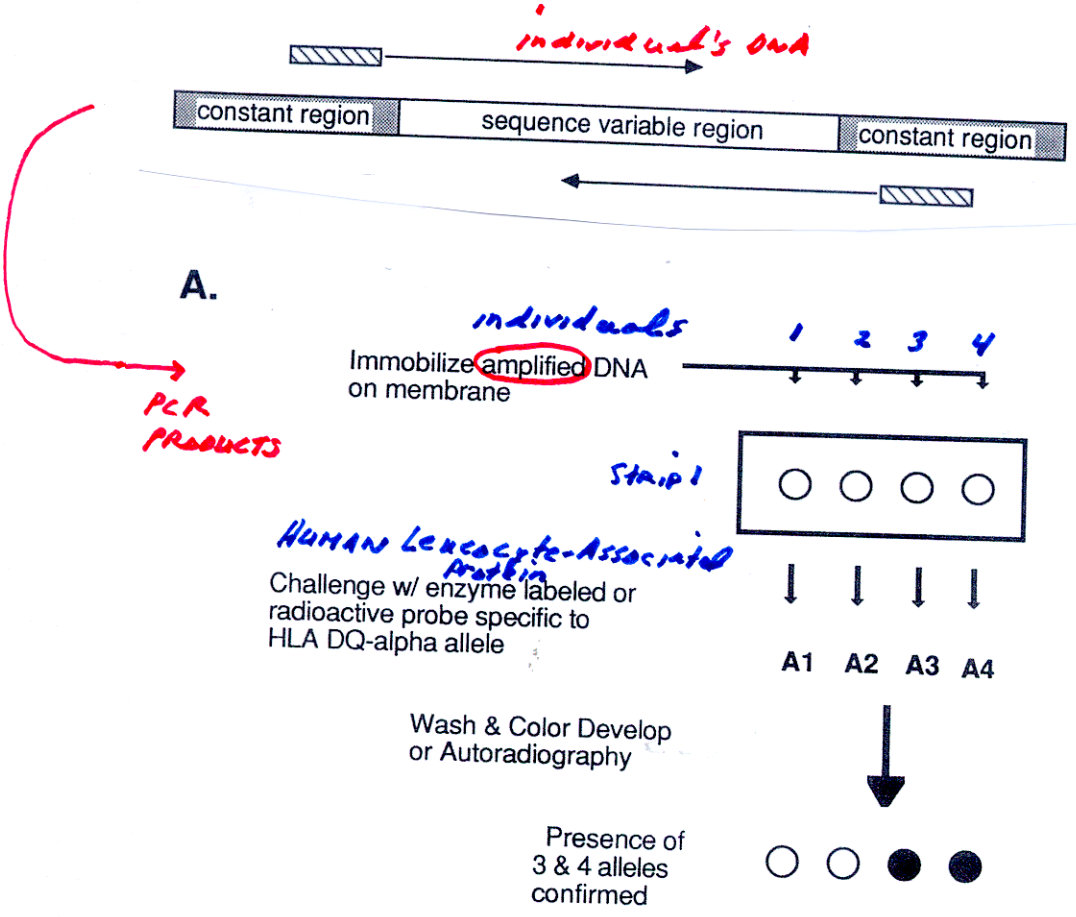
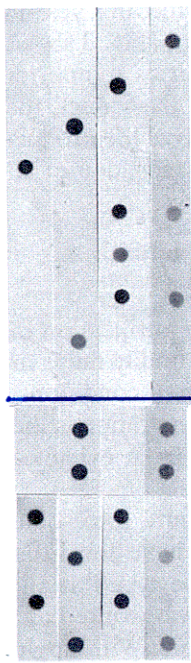


Figure 3

Format for typing amplified DQα gene DNA. (*A*) Steps in the immobilization and detection of amplified DQα alleles; (*B*) prototypic typing experiment. Aliquots from multiple samples are spotted in rows, as in *A*. Strips of membrane-containing spots from each sample are cut apart and challenged with the different probes, as in *A*. The developed strips are reassembled to read off the type, as shown in *B*.

..... BUT NOT AS "SPECIFIC" AS VNTR/STR OR RFLP ANALYSIS



SAMPLE	DQa
POSITIVE CONTROL	1,1
POSITIVE CONTROL	4,4
POSITIVE CONTROL	3,3
POSITIVE CONTROL	2,2
BLOOD [S]	1,4
ONE HAIR [V]	1?,4
TEN HAIRS [V]	1,4
ONE SCENE HAIR	3,3
20 DOG HAIRS	N,A
LIQ. AUTOPSY BLOOD	1,3
DRIED AUTOPSY BLOOD	1,3
PANTIES SPERM DNA	4,2
VAG. E. CELL DNA	1,3
VAG. SPERM DNA	4,2
RECTAL E. CELL DNA	1,3

ASO Control
Suspect Blood
Victim's Hair
Crime Hair
Negative Control
VICTIM/Crime Scene
VICTIM/Crime Scene
VICTIM
VICTIM

Figure 4

Case samples typed for DQα alleles. Samples were tested in the format of Fig. 3 using horseradish peroxidase (HRP)-conjugated probes (for discussion, see text; C. Chang, in prep.).

FINGERPRINTING WORKS

being tested and transfer their radioactivity selectively to those fragments.

Finally, the membrane is placed over standard X-ray film. Radiation emitted from the P-32 gradually exposes the film and gives a precise picture of the DNA fragments.

But the process takes time. The P-32 is so weak that this approach is like sitting in your dentist's chair for two weeks to get an X-ray of your molars. And each of the five loci must be exposed sequentially. The ten weeks of waiting for the X-ray film to be exposed accounts for most of the time it takes to complete an RFLP fingerprint.

Once the film is developed, it's inspected by the scientist conducting the test and at least one other expert. In addition, it is scanned into a computer for precise measurement and comparison against known samples of DNA.

If lines and bars from the known and unknown DNA samples don't match, this is conclusive evidence that they came from different people.

If the X-ray codes do match, some experts will argue that they almost certainly came from the same person. And other experts will challenge that conclusion.—*J. S.*

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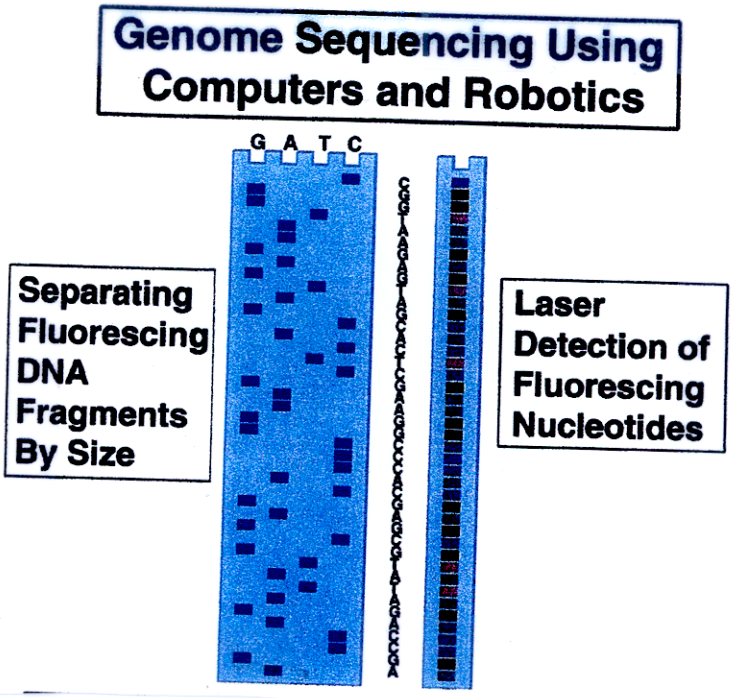
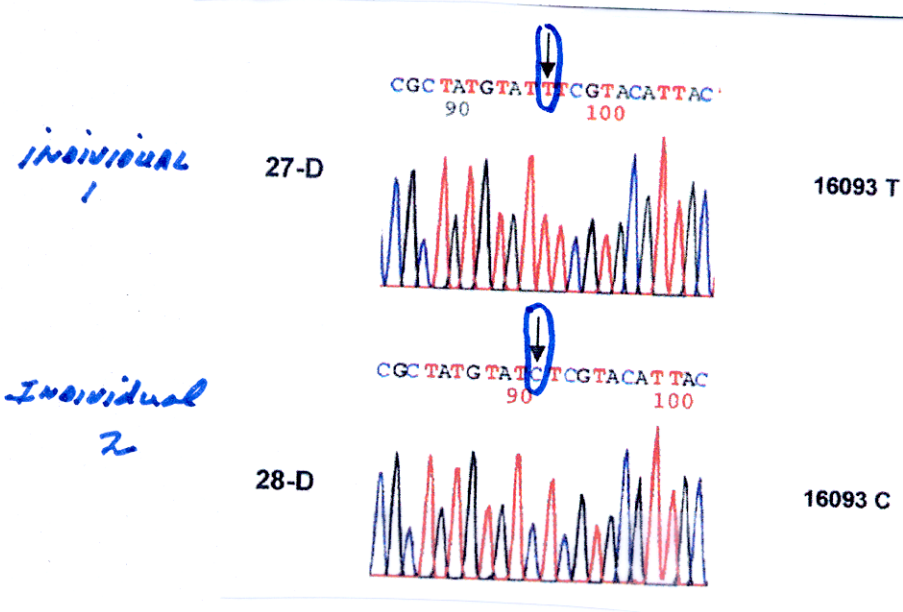
DIRECT DNA SEQUENCING
&
CHIPS TO DETECT
SNPs

Gene & Whole Genome Approaches

Detecting SNPs By Sequencing





Using Chips to Detect SNPs

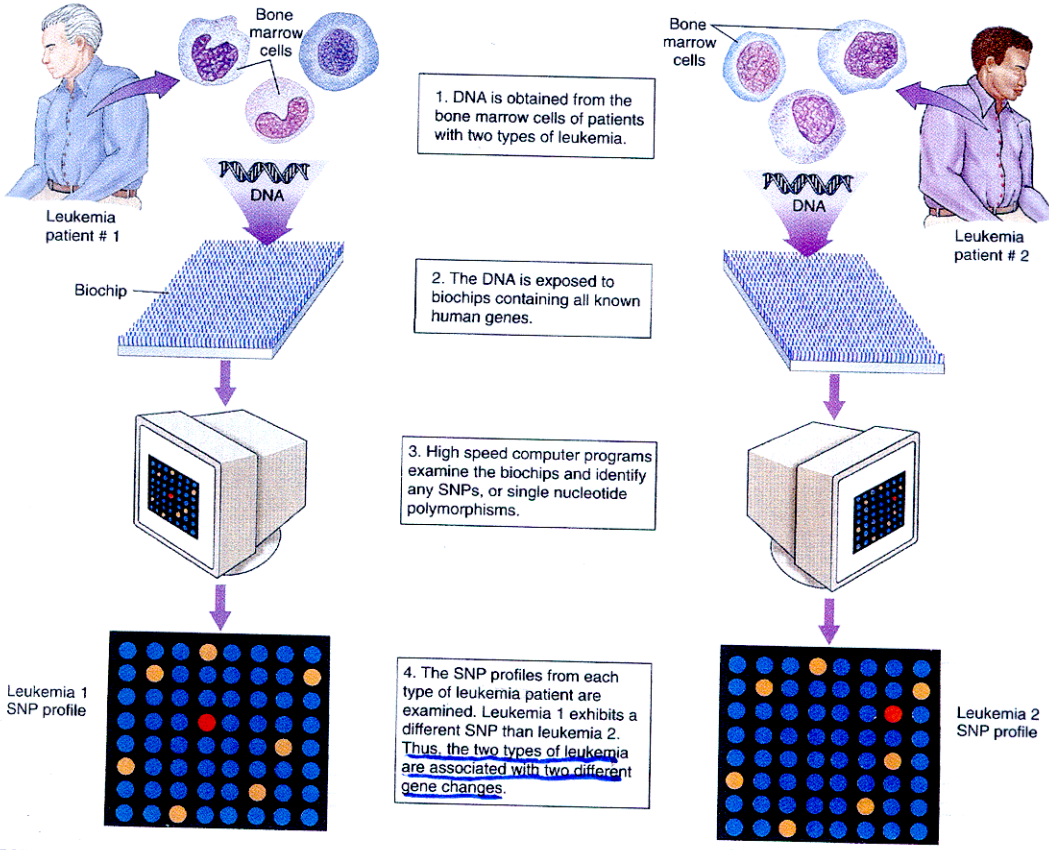


FIGURE 19.16
Biochips can help in identifying precise forms of cancer.

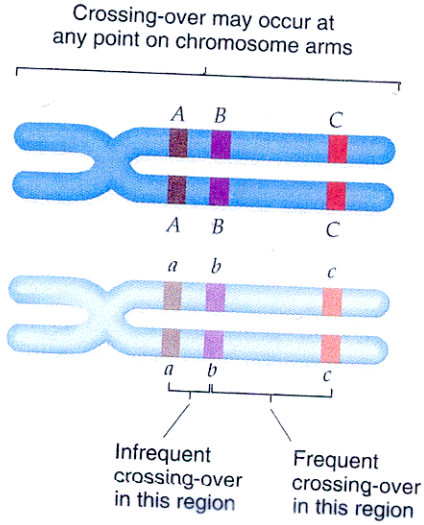
There are Millions of SNPs
That Differ Among
Individuals ....

.... But a small few reflect
our Ancestry & "travel" in
groups on chromosomes —
are linked & may show
specific gene linkages!

CLOSELY-LINKED SNPs ARE INHERITED AS A UNIT

Figure 5.3

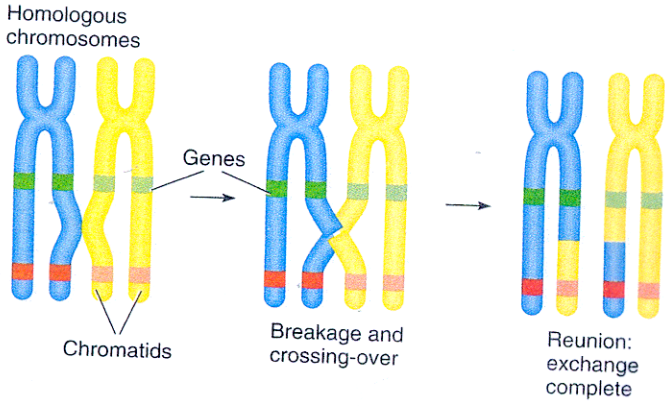
The relationship between recombination and map distance. The farther apart two genes are, the greater the number of possible sites for recombination. Thus, the probability of recombination occurring between genes *A* and *B* is much less than that between genes *B* and *C*. The percentage of recombinants can provide information about the relative genetic distance between two linked genes.



NO CROSSING OVER < 5kb
∴ HAPLOTYPE OF COMPLEX POLYMORPHIC LOCUS

Figure 5.2

Mechanism of crossing-over. A highly simplified diagram of a crossover between two nonsister chromatids during meiotic prophase, giving rise to recombinant (nonparental) combinations of linked genes.



A Haplotype is a closely linked set of specific SNPs

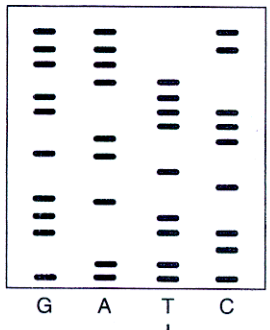
PCR amplification of HLAA locus from one person who is heterozygous for two complex haplotypes

↓

Clone from PCR products

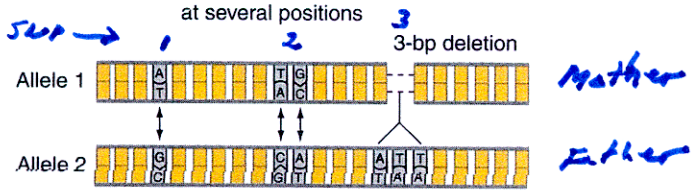
↓

Sequence several clones to obtain at least one sequence from each of the two alleles.



↓

Production of two classes of clones that differ at several positions



3 SNP differences on each chromosome

They are always inherited together & reflect Ancestry!

Figure 9.15 The variations associated with a complex haplotype are best defined by sequencing. Using automated protocols to sequence an entire polymorphic region is often the most rapid and accurate way to detect changes associated with polymorphic alleles at a complex locus.

A Complex Polymorphic Locus with Four Haplotypes

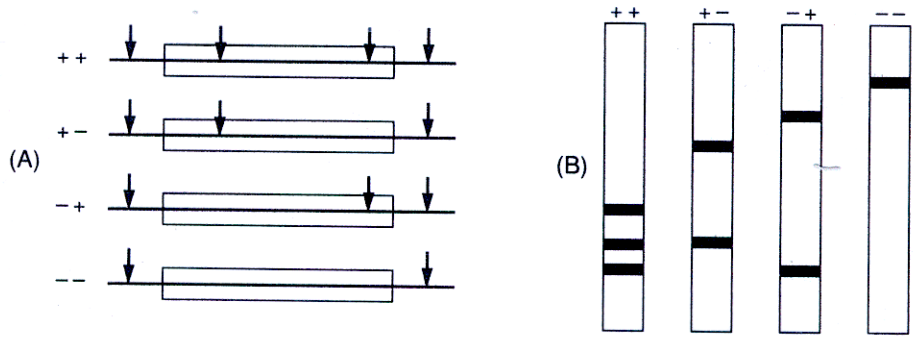


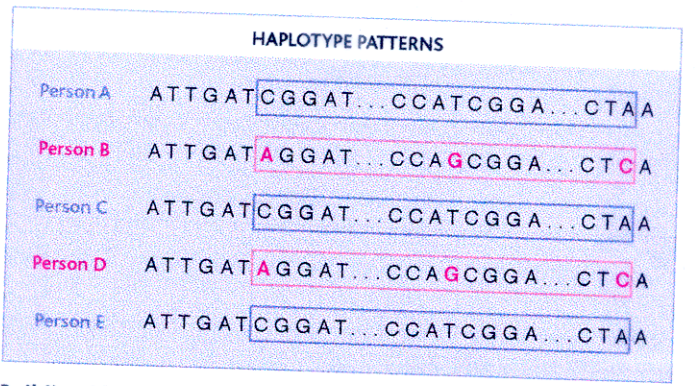
Figure 4–6

A complex polymorphic locus consisting of two adjacent RFLP sites. (A) The four possible haplotypes. Arrows indicate presence of a cleavage site for a restriction endonuclease. Boxed areas are the target sequences recognized by the probe. (B) Southern blots showing the relative electrophoretic mobilities of the fragments produced by restriction enzyme digestion of DNA from each haplotype. Note that all pairwise combinations of the haplotypes can be distinguished from one another; thus, these are codominant alleles.

1
2
3
4

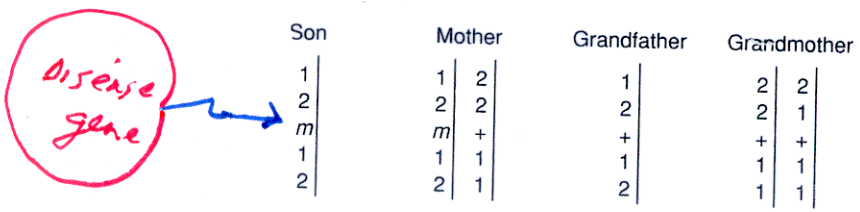
Complex Haplotypes

A contraction of the phrase "haploid genotype," the term **haplotype** refers to a specific combination of linked alleles in a cluster of related genes. Immunogeneticists often use it to describe the combination of alleles of the *major histocompatibility complex (MHC):* a large cluster of genes on human chromosome 6 that play a role in the immune response. With the resolving power to look at DNA at the level of nucleotides, "haplotype" now refers to any set of linked DNA changes along a chromosome. These changes could be in one or several genes, or in noncoding stretches. The **complex** refers to the multiple types of variation that can exist at alternative alleles, including more than one nucleotide substitution, a substitution in combination with a small deletion, duplication, or other insertion. Thus, a **complex haplotype** is a set of linked DNA variations along a chromosome, with the possibility of many differences between alternative alleles.



Building blocks. Persons B and D share a haplotype unlike the other three, characterized by three different SNPs.

Using SNP Haplotypes to identify associations with Disease Genes



∴ these SNPs Associated with Disease Gene

Figure 6–4

Use of haplotypes to identify the source of a new mutation in an X-linked gene. Each column represents a hypothetical haplotype for four RFLP loci, each with two alleles (indicated by 1 or 2); and the disease locus, where + indicates the normal allele and *m* the mutant allele. It is assumed that the presence or absence of the mutant allele can be detected by some direct molecular assay, such as hybridization to an allele-specific oligonucleotide or PCR amplification of a portion of the gene, followed by sequencing. In either case, knowing that the mutation is present in the mother but absent in both of her parents does not tell us which of her parents was the source of the mutant gamete. Haplotype analysis, using closely linked polymorphic loci, solves that problem. In this example, it is clear that the affected boy has his grandfather's X chromosome; therefore, the mutation that he and his mother possess must have originated in his grandfather's germ cells.

The International HapMap Project

The International HapMap Consortium*

*Lists of participants and affiliations appear at the end of the paper

The goal of the International HapMap Project is to determine the common patterns of DNA sequence variation in the human genome and to make this information freely available in the public domain. An international consortium is developing a map of these patterns across the genome by determining the genotypes of one million or more sequence variants, their frequencies and the degree of association between them, in DNA samples from populations with ancestry from parts of Africa, Asia and Europe. The HapMap will allow the discovery of sequence variants that affect common disease, will facilitate development of diagnostic tools, and will enhance our ability to choose targets for therapeutic intervention.

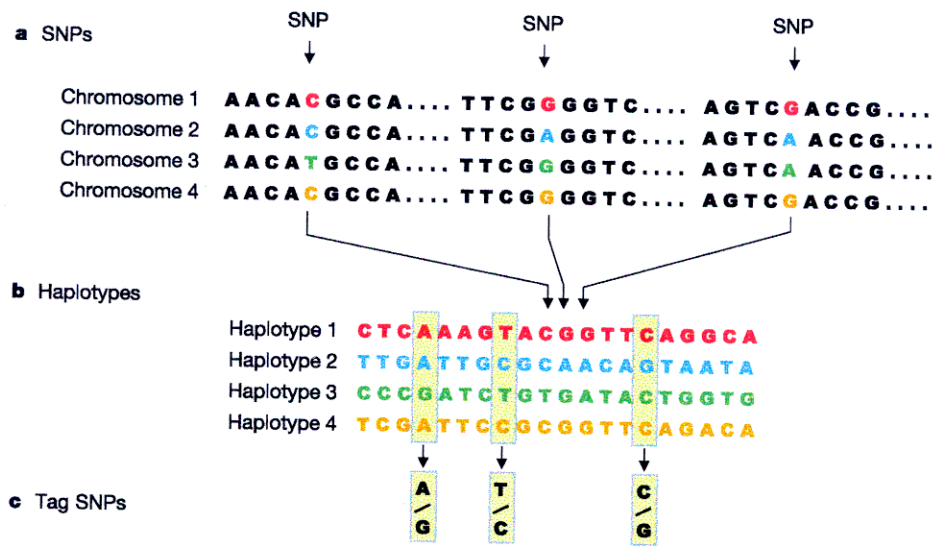


Figure 1 SNPs, haplotypes and tag SNPs. **a**, SNPs. Shown is a short stretch of DNA from four versions of the same chromosome region in different people. Most of the DNA sequence is identical in these chromosomes, but three bases are shown where variation occurs. Each SNP has two possible alleles; the first SNP in panel **a** has the alleles C and T. **b**, Haplotypes. A haplotype is made up of a particular combination of alleles at nearby SNPs. Shown here are the observed genotypes for 20 SNPs that extend across 6,000 bases of DNA. Only the variable bases are shown, including the three SNPs that are shown in panel **a**. For this region, most of the chromosomes in a population survey turn out to have haplotypes 1–4. **c**, Tag SNPs. Genotyping just the three tag SNPs out of the 20 SNPs is sufficient to identify these four haplotypes uniquely. For instance, if a particular chromosome has the pattern A–T–C at these three tag SNPs, this pattern matches the pattern determined for haplotype 1. Note that many chromosomes carry the common haplotypes in the population.

THE 0.1% THAT's Different!

CORRELATE WITH SEQUENCE VARIANTS AFFECTING DISEASE

International HapMap Groups

World view: the HapMap initiative will gather genetic data from African, Asian and ancestrally European populations.

FAR LEFT, L. GEORGIA/CORBIS; LEFT, CORBIS

L; RIGHT, J. SLATER/CORBIS; FAR RIGHT, O. FRANKEN/CORBIS

1. Northern European
2. Western European
3. Yoruba/Nigerian/African
4. Japanese & Han Chinese/Asian

"Group" Genetic Diversity to Disease & other Aspects of Biology

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Ethical Issues Related to the HAPMAP

Box 1

Community engagement, public consultation and individual consent

As no personally identifiable information will be linked to the samples, the risk that an individual will be harmed by a breach of privacy, or by discrimination based on studies that use the HapMap, is minimal. However, because tag SNPs for future disease studies will be chosen on the basis of haplotype frequencies in the populations included in the HapMap, the data will be identified as coming from one of the four populations involved, and it will be possible to make comparisons between the populations. As a result, the use of population identifiers may create risks of discrimination or stigmatization, as might occur if a higher frequency of a disease-associated variant were to be found in a group and this information were then overgeneralized to all or most of its members[64]. It is possible that there are other culturally specific risks that may not be evident to outsiders[65]. To identify and address these group risks, a process of community engagement, or public consultation, was undertaken to confer with members of the populations being approached for sample donation about the implications of their participation in the project[66,67]. The goal was to give people in the localities where donors were recruited the opportunity to have input into the informed consent and sample collection processes, and into such issues as how the populations from which the samples were collected would be named. Community engagement is not a perfect process, but it is an effort to involve potential donors in a more extended consideration of the implications of a research project before being asked to take part in it[68]. Community engagement and individual informed consent were conducted under the auspices of local governments and ethics committees, taking into account local ethical standards and international ethical guidelines. As in any cross-cultural endeavour, the form and outcome of the processes varied from one population to another. A Community Advisory Group is being set up for each community to serve as a continuing liaison with the sample repository, to ensure that future uses of the samples are consistent with the uses described in the informed consent documents. A more detailed article discussing ethical, social and cultural issues relevant to the project, and describing the processes used to engage donor populations in identifying and evaluating these issues, is in preparation.

SNPs Should Be Powerful Indicators of Disease Susceptibility

D528–D532 Nucleic Acids Research, 2004, Vol. 32, Database issue
DOI: 10.1093/nar/gkh005

SNP500Cancer: a public resource for sequence validation and assay development for genetic variation in candidate genes

Bernice R. Packer*, Meredith Yeager, Brian Staats, Robert Welch, Andrew Crenshaw, Maureen Kiley, Andrew Eckert, Michael Beerman, Edward Miller, Andrew Bergen[1], Nathaniel Rothman[1], Robert Strausberg[2] and Stephen J. Chanock[3]

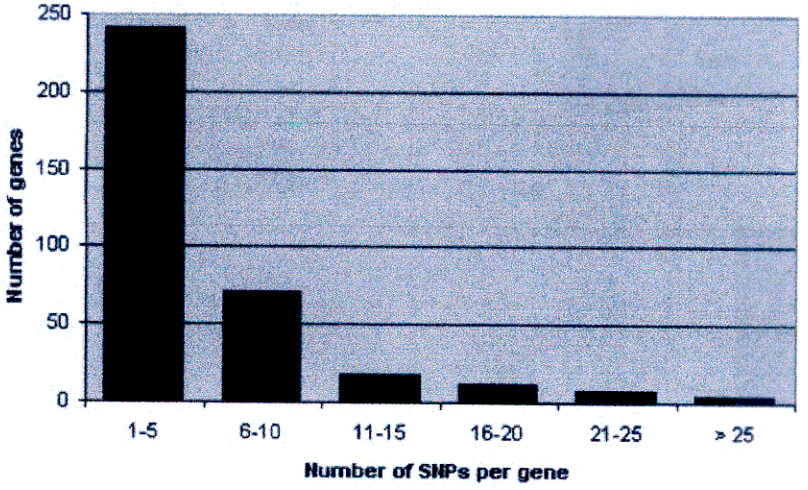


Figure 1. SNPs per gene.

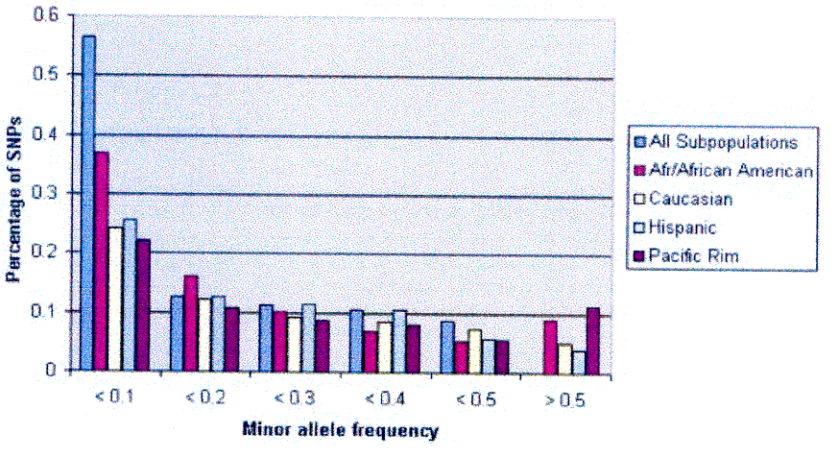
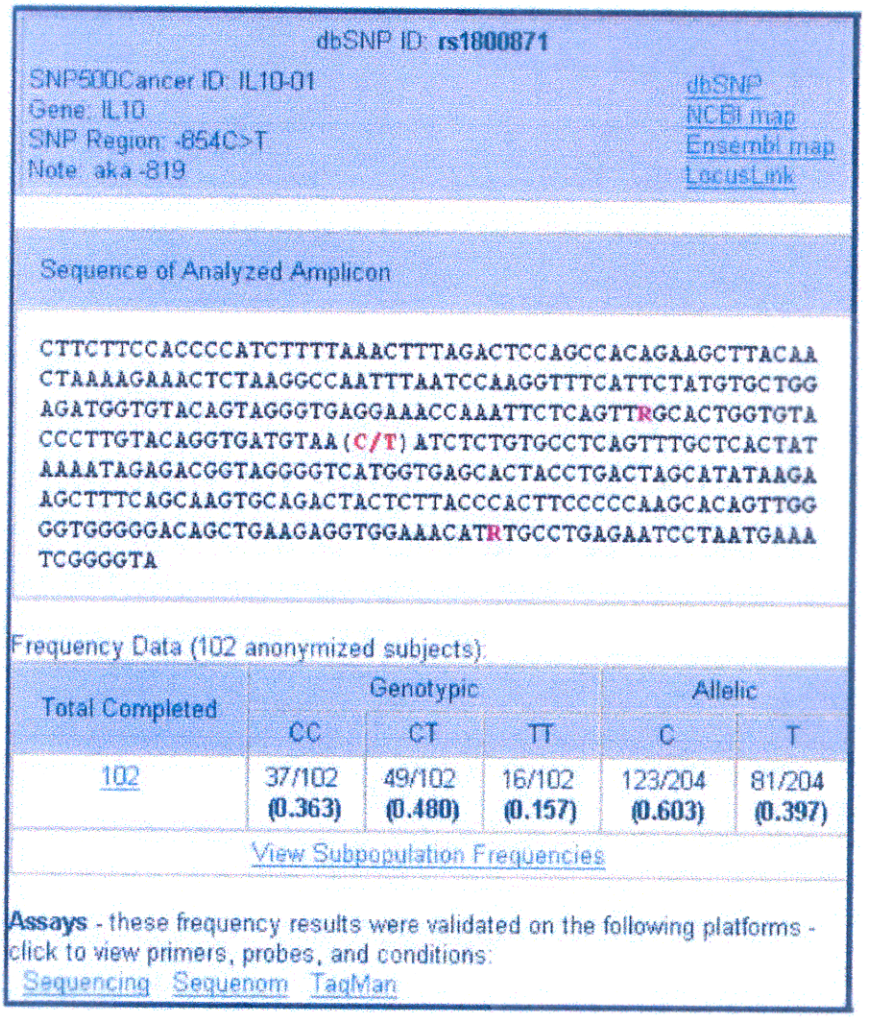


Figure 2. SNP500Cancer allele frequencies by subpopulation.

SNPs Found in IL10 Gene



dbSNP ID: **rs1800871**

SNP500Cancer ID: IL10-01
Gene: IL10
SNP Region: -854C>T
Note: aka -819

dbSNP
NCBI map
Ensembl map
LocusLink

Sequence of Analyzed Amplicon

CTTCTTCCACCCCATCTTTTAAACTTTAGACTCCAGCCACAGAAGCTTACAA
CTAAAAGAAACTCTAAGGCCAATTTAATCCAAGGTTTCATTCTATGTGCTGG
AGATGGTGTACAGTAGGGTGAGGAAACCAAATTCTCAGTTRGCACTGGTGTA
CCCTTGTACAGGTGATGTAA(C/T)ATCTCTGTGCCTCAGTTTGCTCACTAT
AAAATAGAGACGGTAGGGGTCATGGTGAGCACTACCTGACTAGCATATAAGA
AGCTTTCAGCAAGTGCAGACTACTCTTACCCACTTCCCCCAAGCACAGTTGG
GGTGGGGGACAGCTGAAGAGGTGGAAACATRTGCCTGAGAATCCTAATGAAA
TCGGGGTA

Frequency Data (102 anonymized subjects):

Total Completed	Genotypic			Allelic	
	CC	CT	TT	C	T
102	37/102 **(0.363)**	49/102 **(0.480)**	16/102 **(0.157)**	123/204 **(0.603)**	81/204 **(0.397)**

View Subpopulation Frequencies

Assays - these frequency results were validated on the following platforms - click to view primers, probes, and conditions:
Sequencing Sequenom TaqMan

Figure 4. SNP information.

SNP Database

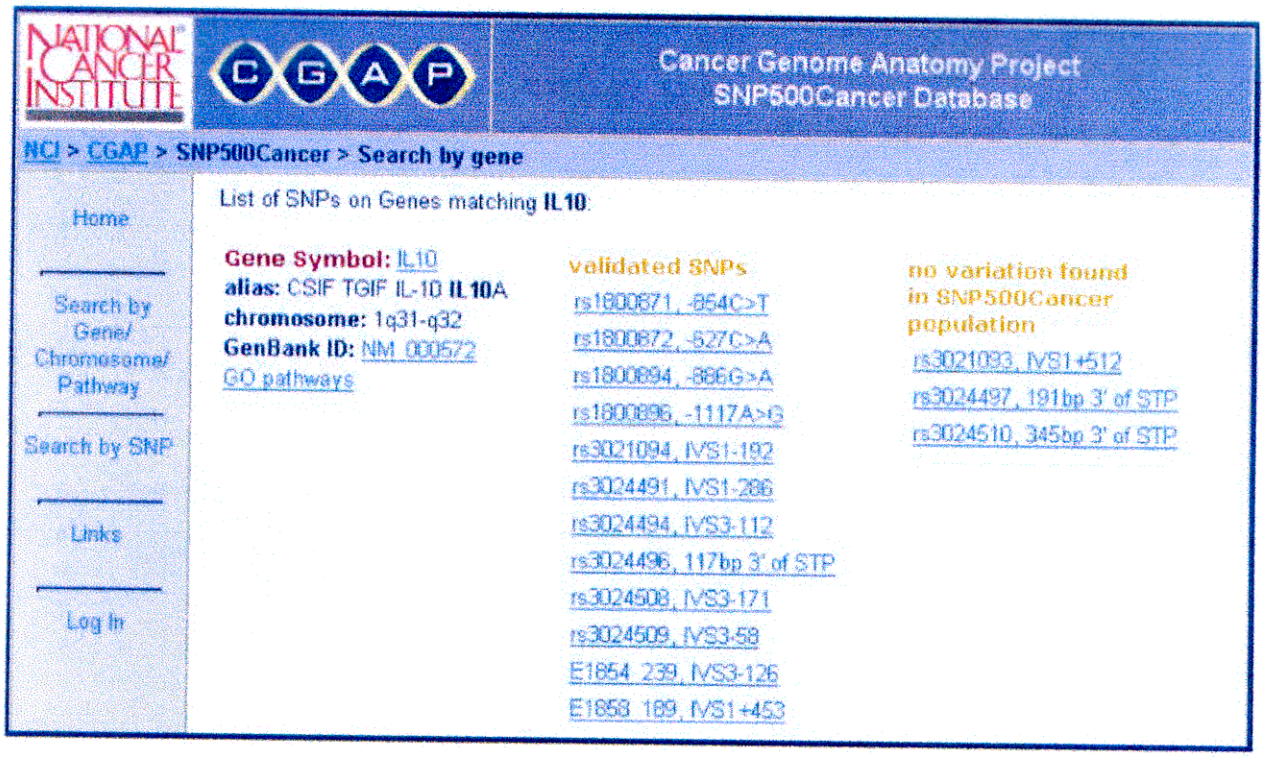


Figure 3. Listing a gene's SNPs.

Frequency Data (102 anonymized subjects)

dbSNP ID: rs1800871

Subpopulations	Genotypic CC	Genotypic CT	Genotypic TT	passed *HWE*?	Allelic C	Allelic T
Total Completed	37/102 **(0.363)**	49/102 **(0.480)**	16/102 **(0.157)**	-	123/204 **(0.603)**	81/204 **(0.397)**
Afr/Afr American	5/24 **(0.208)**	14/24 **(0.583)**	5/24 **(0.208)**	passed	24/48 **(0.500)**	24/48 **(0.500)**
Caucasian	15/31 **(0.484)**	14/31 **(0.452)**	2/31 **(0.065)**	passed	44/62 **(0.710)**	18/62 **(0.290)**
Hispanic	13/23 **(0.565)**	8/23 **(0.348)**	2/23 **(0.087)**	passed	34/46 **(0.739)**	12/46 **(0.261)**
Pacific Rim	4/24 **(0.167)**	13/24 **(0.542)**	7/24 **(0.292)**	passed	21/48 **(0.438)**	27/48 **(0.563)**

Figure 5. Genotypic and allelic frequencies for a SNP.

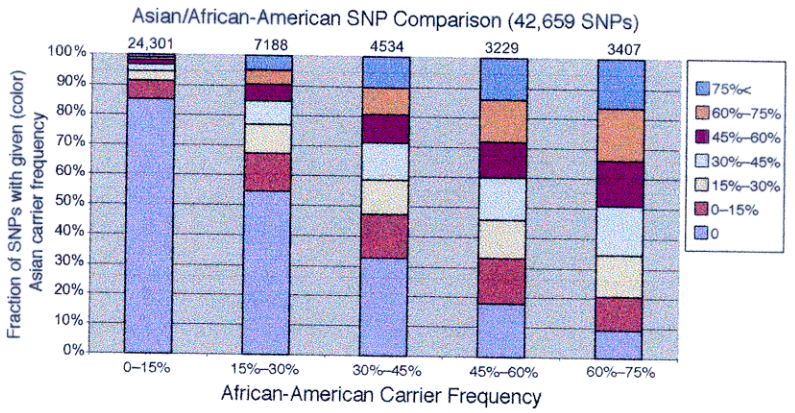
SNPs Might Be Useful for PROVIDING CLUES AS TO DISEASE Genes

NEWS

Race and Medicine

Genetic studies of population differences, although controversial, promise clues to disease as well as new drug targets, scientists believe

24 OCTOBER 2003 VOL 302 SCIENCE www.sciencemag.org



Biodiversity. More than 42,000 SNPs (genetic variations) found in African Americans are divided into columns according to how frequently they appear in that population. Colors indicate the frequency with which these same groups of SNPs are found in East Asians. For instance, in the second column, of the 7188 SNPs that are found in 15% to 30% of African Americans, more than half show no variation in Asians.

NOTE: Diversity OF Alleles & Frequency Differences

USING GROUP/"RACE" GENE VARIABILITY DATA