Genetic and Physical Mapping

The ultimate goal of mapping is to identify the gene(s) responsible for a given phenotype or the mutation responsible for a specific variant. The initial steps in mapping are to:

1. establish the proximity of genes or traits to one another
2. assign the genes to a particular chromosome

What is the difference between a genetic and a physical map?

**Genetic maps** depict relative positions of loci based on the degree of recombination. This approach studies the inheritance/assortment of traits by genetic analysis.

**Physical maps** show the actual (physical) distance between loci (in nucleotides). This approach applies techniques of molecular biology.
Rules for the first genetic (i.e., linkage) map were uncovered by Mendel. Mendel’s Law of Independent Assortment

If traits segregate independently of one another, they are “unlinked.”

Enter Punnett, Bateson and Saunders (1905), who examined two other traits (flower color & pollen shape) in pea plants.

If traits are co-inherited more often than expected by chance, they are “linked.”
Morgan & Sturtevant (1913):
Recombination frequency is a measure of the distance between two genes

See Box 5.2, Brown p.141; example of a multi-point cross

Genetic mapping via Linkage Analysis

By doing series of crosses, increasing the number of genetic markers (and examining large numbers of progeny to detect rare crossing-over events):

- Various linkage relationships appear (complete, partial, independent assortment)
  Between two loci, range from 0 - 0.5 (0 - 50 cM)
- The number of linkage groups emerge

What is the relationship between number of linkage groups and chromosomes?
Genetic mapping via Linkage Analysis

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What is the relationship between number of linkage groups and chromosomes?
Need they always be the same?

How is it possible to assign traits (or a linkage groups) to a specific chromosome?

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Easy for sex-linked traits, but what about autosomes?

What is the advantage of a test cross over a dihybrid cross for linkage mapping?
Genetic mapping relies on polymorphic markers/traits

Construction of a genetic linkage map in man using restriction fragment length polymorphisms.

Botstein D, White RL, Skolnick M, Davis RW.

We describe a new basis for the construction of a genetic linkage map of the human genome. The basic principle of the mapping scheme is to develop, by recombinant DNA techniques, random single-copy DNA probes capable of detecting DNA sequence polymorphisms, when hybridized to restriction digests of an individual's DNA. Each of these probes will define a locus. Loci can be expanded or contracted to include more or less polymorphism by further application of recombinant DNA technology. Initially polymorphic loci can be tested for linkage relationships in human pedigrees by established methods; and loci can be arranged into linkage groups to form a true genetic map of the DNA marker loci. Pedigrees in which inherited traits are known to be segregating can then be analyzed, making possible the mapping of the gene(s) responsible for the trait with respect to the DNA marker loci, without requiring direct access to a specified gene's DNA. For inherited diseases mapped in this way, linked DNA marker loci can be used predictively for genetic counseling.

Linkage Mapping in Humans: Association of disease state with a minisatellite (Mn) polymorphism

[Diagram of genetic linkage map]
Genetic Mapping in Bacteria

What are the limitations to constructing a genetic map?

- Access to polymorphic traits or markers
- Need for a large number of progeny and/or multiple generations
- Best performed in model organisms subject to selective breeding
- Crossing-over does not occur at random (maps of limited accuracy)

In contrast, some form of physical map can be constructed for any organism.
Physical Mapping of Genomes

Physical maps plot the actual location of DNA sequences in the genome

**Restriction maps**: locate the positions of and distances between endonuclease recognition sites on a DNA molecule.

**Long-range restriction maps**: locate the positions of rare-cutting endonuclease recognition sites on a DNA molecule by PFGE.

**Clone (contig) maps**: consist of libraries of overlapping clones where the relationship of each clone to other clones has been resolved.

**Fluorescent in situ hybridization (FISH)**: locates the position of a marker by hybridizing a labeled probe to intact chromosomes.

**Optical maps**: visually inspects and measures the positions of endonuclease recognition sites on a DNA molecule.

**EST (expressed sequence tags) maps**: plot the location of transcribed sequences.

**STS content maps**: (tbd)

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Restriction mapping:

- **finding the distance between restriction enzyme recognition sites in a DNA fragment**

  1. Combination of single & double digests

  2. Partial digestion such that DNA is not cut to completion

How could one orient these fragments without relying on complete digests?
Long-range restriction mapping

Two innovations:

1. Pulsed-field gel electrophoresis (for separating large DNA fragments)

2. “Rare-cutting” restriction enzymes

8-bp recognition sites: e.g., NotI (GC^GGCCGC); SwaI (ATTT^AAAT); Pmel (GTGT^AAAC)

Homing (intron- or intein-encoded) endonucleases: e.g., I-CeuI (TAACCTAAGGTGCT^CTAA)

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Optical Mapping of Chromosomes I

Figure 1
Staining in optical mapping. Large DNA molecules, comprising bacterial chromosomes (a-e) or artificial chromosomes, yeast artificial chromosomes (f), or genomic DNA (g), are mounted on a planar glass surface (g). The DNA molecules are digested with an restriction enzyme, cleavage sites are visible as small gaps between DNA fragments, which retain their original order (b). The DNA is stained with an intercalating dye and visualized by fluorescence microscopy (a-d). Digital images are recorded with a charge coupled device camera. The fragments are scored by measuring the integrated fluorescence intensity and maps of single molecules are constructed (e-f). Fragments as small as 100 bp can be imaged and sized. Continuous maps of cloned DNA are created using a publishable scheme (g) and maps of genomic DNA are aligned into consensus using the DGHG software (h). Consensus maps are subsequently used as scaffolds for sequence assembly and verification.

Optical Mapping of Chromosomes II
Optical Mapping of Chromosomes III

Fluorescence in situ hybridization I

Two-color detection: Hopman et al. 1986, Histochemistry 85: 1
Three-color detection: Nederlof et al. 1989, Cytometry 10: 20
Combinatorial color-coding (mFISH): Nederlof et al. 1990, Cytometry 11: 126
Somatic cell hybrids and radiation hybrid mapping

Figure 1.2. Radiation hybrid mapping. The objective of radiation hybrid (RH) mapping is to generate a panel of clones, each carrying a different set of fragments of the genome being mapped. Hybridization of labeled cells causes the chromosomes to fragment. In the cells, they are fused with a Chinese hamster cell line that lacks the gene for the homologous RH fragment, which allows cells that take up chromosome fragments, one of which encodes TK, to be selected by selecting for TK expression. Such radiation hybrid cell lines are then treated to have fragments either donor or recipient, up to several MB in length, these fragments are then incorporated into the hamster chromosomes or segregated as exact chromosomes.
A common language for physical mapping the human genome

1. What is an STS?
2. How many people read the assigned (2-page) article?
3. Why does an STS need to be a unique sequence?
4. How will STS technology “solve the problem of merging data from many sources”? (And what kind of data are they talking about?)
5. How does one find an STS in the genome?
6. How will STSs assist in the assembly of contig maps?
7. What are some of the problems in developing contig maps?
8. What are some of the disadvantages of restriction maps?
9. How many STSs are needed to be useful?
10. What are the advantages of using STSs as genomic landmarks?
11. How many STSs are needed to be useful?
Assembling clone contigs by STS content mapping

STS content mapping assays for the presence of known sequences (STS) in DNA fragments from any source (clones, RH, etc.) and can thereby align physical maps derived by different methods.

Another method for mapping a gene/clone is to obtain pure preparations of a particular chromosome by flow cytometry or fluorescence-activated chromosome sorting.