Genome Projects

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Outline

- What are genomes, and how were they sequenced?
- What can we do with genomes?
- How do we look at genomes?
- How do we choose a browser?
- How do the browsers work?

Why Study Genomes?

- Understand biological processes
- Understand pathological processes
- Diagnose, prevent, and cure diseases

So what is a genome?

- A genome is the full collection of genetic material (DNA) of an organism (including non-nuclear DNA, such as mitochondrial or chloroplast DNA)
- It is more than the protein coding genes (which are only a small percentage of the human genome)
- In humans there are 3,000,000,000 base pairs of DNA in the haploid genome

What else is in the DNA? (aside from genes)

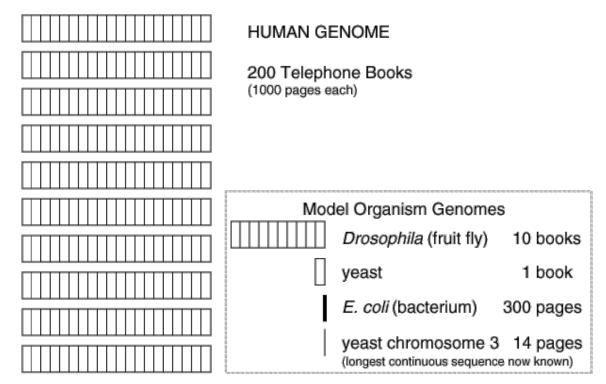
- There are areas that don't code for genes at all
- There are areas that regulate the genes
 - When should a protein be expressed? night/day, fetus/adult
 - Where should a protein be expressed?
 eye, lung, muscle, brain

AGGTTAGATTATGCCCCGAGGGCGCCCCAGCCGAAATTTTTAATGCAGGTTTAATAGTTTAGAGC CTGTGGGGCTTCCATGGCTTGGTTCTGCTGTTCTTCACTGGGGGACTTGGGGGGGCCCTGGGAGCTTG TGATGGGGCCTGTCTCCACCTCTGTAAATCCAAGGAGTCAGATGACAAATCTGTCATTTCGGGCG ACACACTCCCCTGAGGAAAGGGCCTTGCAGGAGGGCAGAGCAGCTTGCTGGGCATGGCAGGGAGT GGGGCCTGCAGGAGCCCCTGTGTGTGCCAGCCCTCCCCTGCCAGCATCCCCAGGAGGCCCC CAGGGCAGGTAAGTGCCAGGTCCCCCCTCAGCTCACCGTTGTCCTTCCCCCTTGACGAACGCCTCC CACTCCCGGAACCACTGCATGCTGATGCAGTAGATGACGCCCGGCGACTCCTCGGCCTGGAAGGC TCAGCAAGGATTTCCCCAAATGCCCCCGCCCCAGTCCCTCACCCAAGAGTCTTACAAAAACACCAG AGAGGACGGTGGTAACATTTTGTGGGGTCTCTTCAATAACTGTTTGACCCCAACTGAGACCACAAGG GAGATTCTACTTTTGAGAAGGAATCTCACTCTGTCACCCAGGCTGGAGTGCAGTGGCGCGATCT CGGCTTACTGCAACCTCTGCCTCCCAGGTTCAAGCGATTCTCCCCACCTCAGCCTCTTGAGTAGCT GGGATTACAGGTGTGTGCCACCACCTGGCTACTTTTTGTGTTTTTAGTAGAGACGGGGTTTCGCC ATGTTGGCCAGGCTGGTCTTGAACTCCTGACCTCAGGTGATCCGCCCACCTCGACCTCTCAAAAG TGCTGGGATAACAGGCATGAACCACTGCGCCCGGCCTGGGAGATGCTAATTTTCTCCCGGTTGAAT AGAATGTGCCTATCTGCTCAGAGAGGCAGCTCTCCTTCTGACAGGAGCATTTTCTTTTCGAGAG TGACCTCCTGGGCTCAAGTGATCCTCCCACCTCAGCCTCCTGAGTAGGTTGGACCACAGGTGCAT ACCACTAGGCCCAGCCCTGACAGTCTCTTTTTCGTTTGTGTGTTCTGAGACAGGGTCTCACTCTATT GCCCAGGCTGCGGTGCAGTGGCATGATCACGGCTCACTGCAGCCTCAACCTCCCAGGCTTAGGTG ATCCTCCCAACTCACTCAGCCCTCCAGGTAGCGGGGGCTACAGGTACACATCACCATGCCTGGCT AATTTTTGTATTGTTTGTAGAGATGGGGGTTTCGCCATGTTGGCCAAGTTGGTCTTGAACTCCTGG

Challenges of DNA

- DNA has only four letters
- They are strung together with NO obvious punctuation
- There are no signals to say "a gene starts (or ends) here"
- How do we make sense of so much information?

ORNL-DWG 91M-17472



If compiled in books, the data would fill an estimated 200 volumes the size of a Manhattan telephone book (at 1000 pages each), and reading it would require 26 years working around the clock.



The Human Genome Project

- Was planned in 1988, started in 1990
- Originally planned to take 15 years, in three five year stages

Objections to the genome project

1) Fear that funding will be diverted from other areas of research

2) What is the value of sequencing a complete genome, given the high proportion of nongenic sequences ("Junk DNA").

Two alterations in the original plan helped:

1) Focus shifted from large-scale sequencing to mapping the genome, which would hasten the search for disease genes

2) Simultaneously determine the nucleotide sequence of the genomes of other organisms; this provides comparisons and points of reference for the human sequence

Stage 1 1991-1995

- Creating a Genetic Map of the genome
- Creating a Physical Map of the genome
- Creating a set of overlapping clones
- Create faster/cheaper methods of sequencing
- Create software/databases that can deal with the data

Stage 2 1994-1998

- Finish mapping (both genetic and physical)
- Start sequencing
- Start annotation gene finding and placement on maps

Stage 3 1998-2003

Area Genetic map	Goals 1993-98 Average 2- to 5-cm	Status as of Oct. 1998 1 cm map published Sept. 1994	Goals 1998-2003 Completed
Physical map	Map 30,000 STSs	52,000 STSs mapped	Completed
DNA sequence	Complete 80 Mb for all organisms by 1998	180 Mb human plus 111 Mb non-human	Finish 1/3 of human sequence by end of 2001 Working draft of remainder end of 2001 Complete human sequence by end of 2003
Human sequence Not a goal variation		-	100,000 mapped SNPs
Gene Identification	Develop Technology	30,000 ESTs mapped	Full-length cDNAs
Functional analysis	Not a goal	-	Develop genomic-scale technologies

Techniques

The major breakthrough that allowed the Human Genome Project to take off were two techniques:

 Improvements in sequencing: Cycle Sequencing Automated Sequencers Flourescent Dyes High Throughput - lower costs

2) PCR

Markers

There are many different types of markers:

RFLP: Restriction Fragment Length Polymorphism

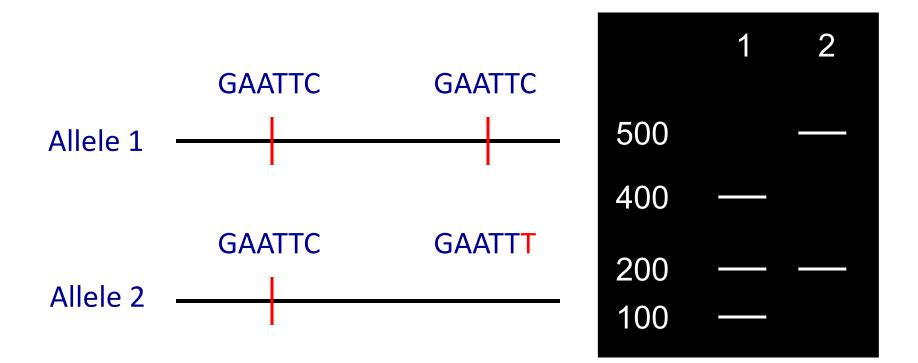
Microsatellite

VNTR: Variable Number Tandem Repeat

STS: Sequence Tagged Site

Restriction Fragment Length Polymorphism

700 base pair PCR fragment



Microsatellites

Microsatellites are small repetitive stretches of DNA, usually repeats of di, tri and tetra nucleotides.

For example CACACA, or CAGCAGCAG.....

Because these stretches repeat, when the DNA recombines, its very easy for the machinery to "slip" and add or subtract a few copies

Recombination Parent chromosomes

AAAACAGCAGCAGTTTTT AAAAAAGAAGCAGCAGCAGTTTTT

Daughter chromosomes

- Allele 1 AAAACAGCAGCAGCAGTTTTT
- Allele 2 AAAACAGCAGTTTTT

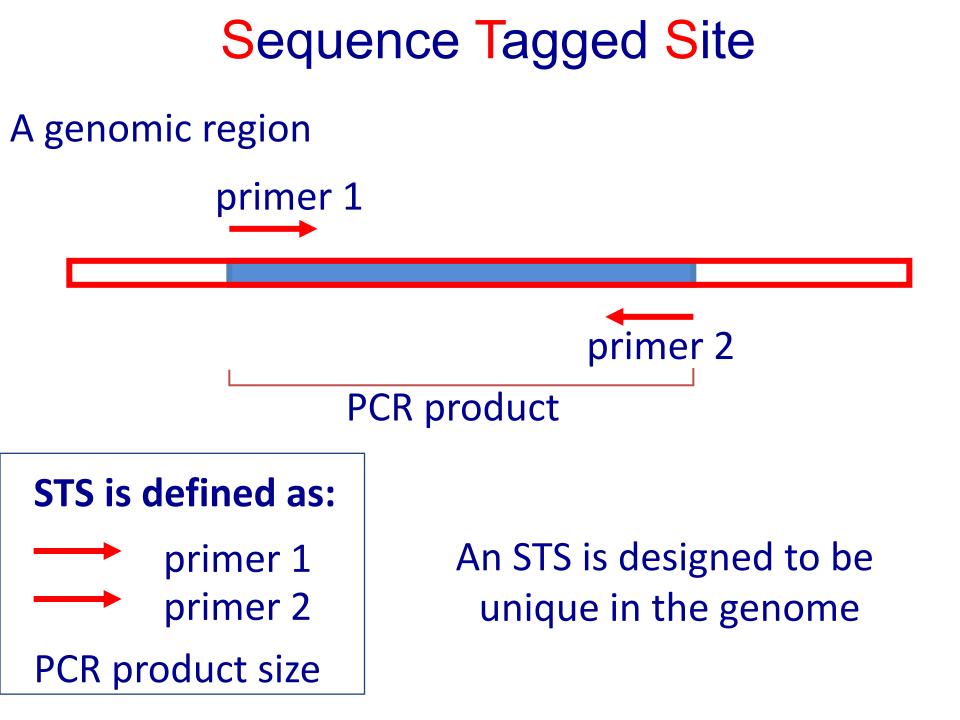
Allele

of repeats

Variable Number Tandem Repeat

Regions of DNA that repeat - for example, microsatellites, although VNTR's can have more complex sequence.

This is also detected by performing PCR and looking at the number of repeats on a gel



Maps

There are two types of maps:

Genetic - measures recombination distance

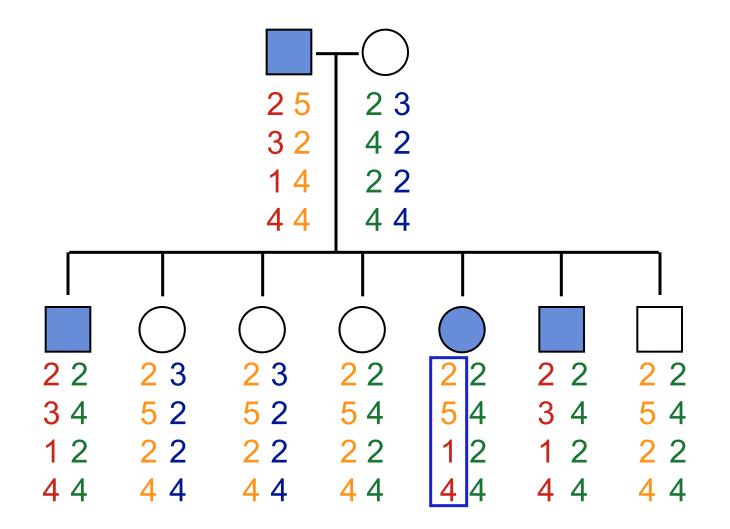
Physical- measures physical distance

Genetic Maps

A genetic map measures recombination distance and answers the question, "How often are two markers found together?"

Two markers are said to be 1 centiMorgan (cM) apart if they are separated by recombination 1% of the time. A genetic distance of 1 cM is roughly equal to a physical distance of 1 million basepairs (1 Megabase or Mb)

Genetic Maps



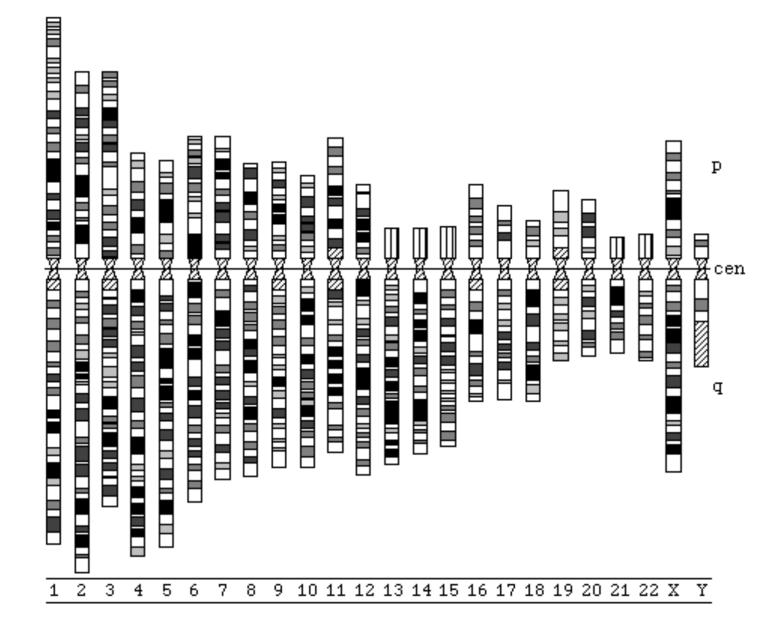
Physical Maps

Physical maps vary greatly. The lowest resolution map are the chromosome banding patterns (ideogram).

The highest resolution map is the actual sequence.

What we actually use is in between. One type of map developed as part of the genome project is the radiation hybrid map (RH map)

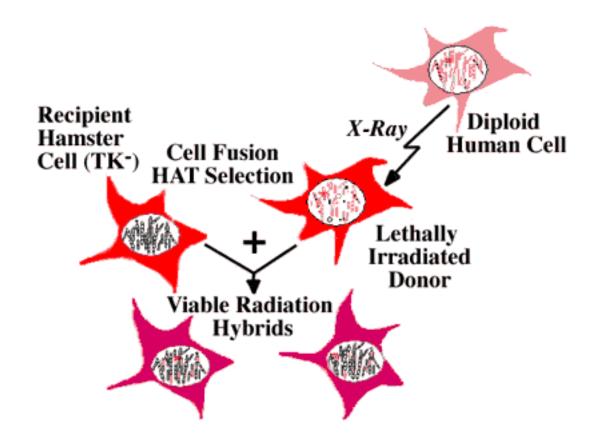
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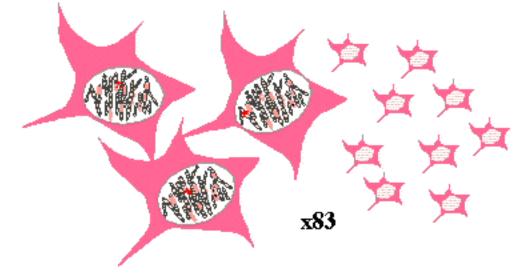
"Whole Genome" Radiation Hybrid Mapping



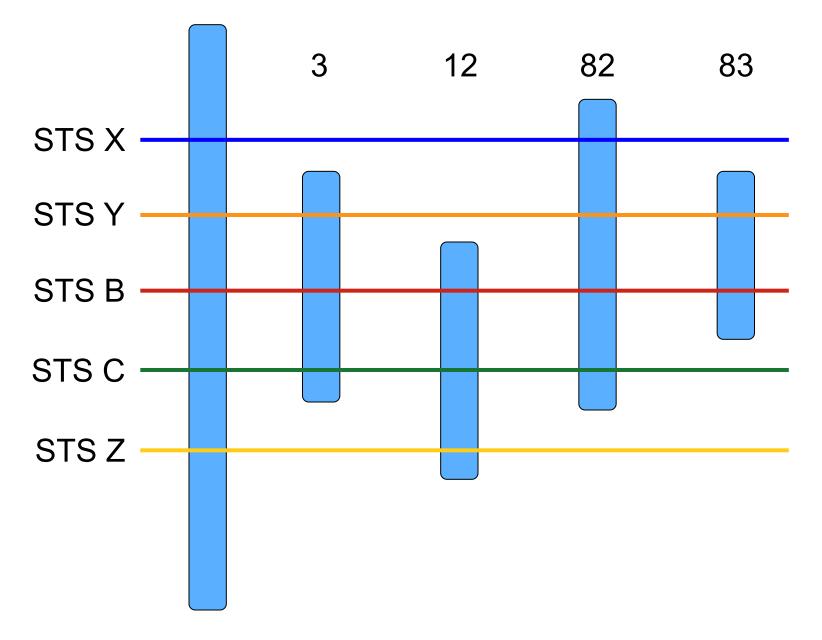


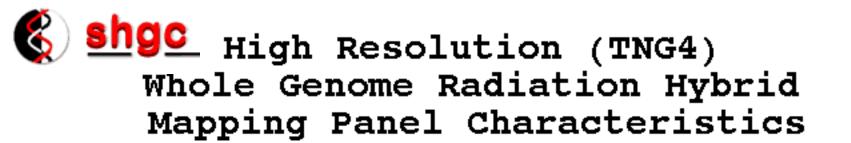
Scoring STSs on a Radiation Hybrid Panel

Radiation Hybrid Panel



Chromosome

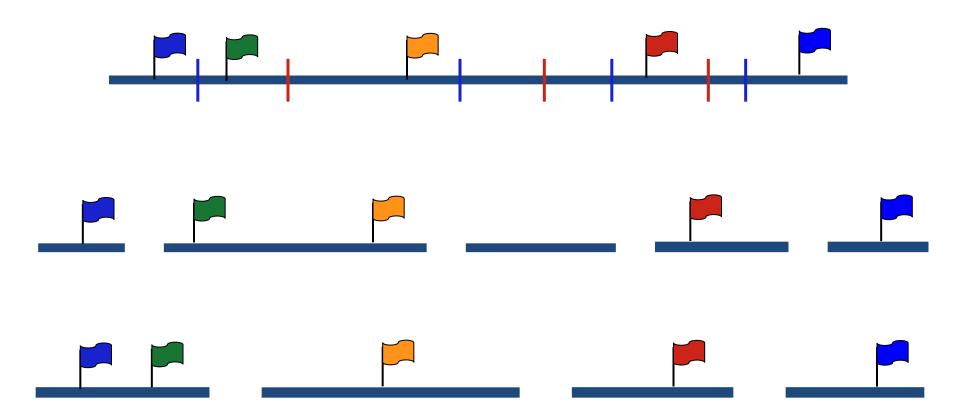


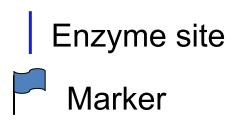


Number of hybirds in mapping panel	90
Fraction of human genome retained in each hybrid	0.16
Average size of human fragments	800 kb
Relationship of X-ray breakage to distance	1% breakage = 4 kb
Average resolution of comprehensive map	60 kb
Average resolution of 1000:1 map	100 kb

How was the genome cloned?

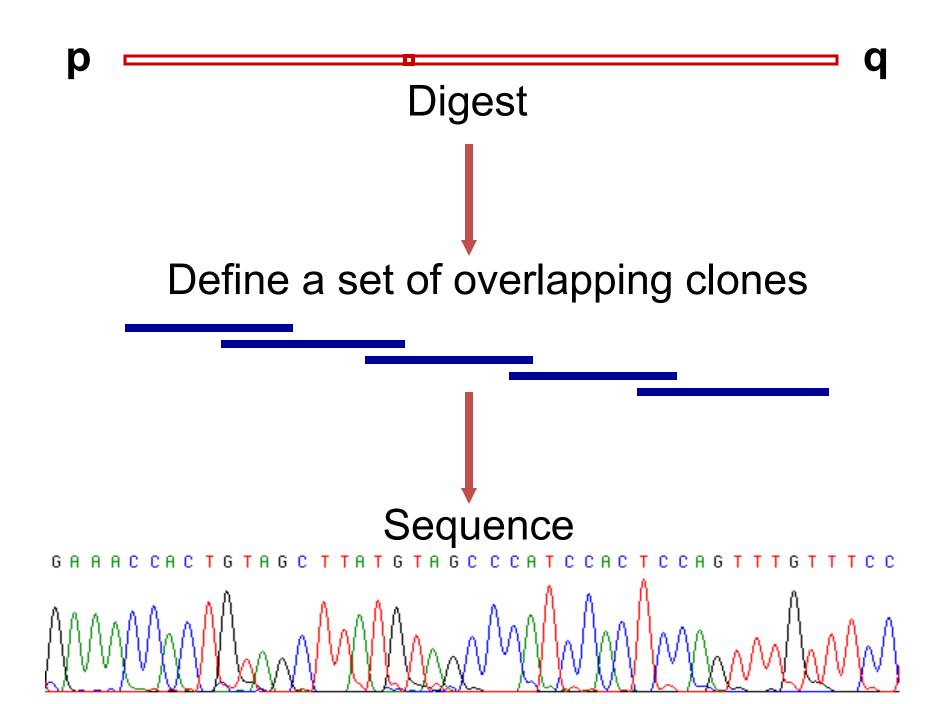
First rare cutting enzymes were used to generate pieces ~150,000 base pairs long. Different enzymes were used to get overlapping segments.





BACs (Bacterial Artificial Chromosomes) were placed along the map with the help of markers, and the least redundant path was chosen.

Then each BAC was broken down the same way, the fragments were placed in order using restriction enzyme mapping, and sequenced.

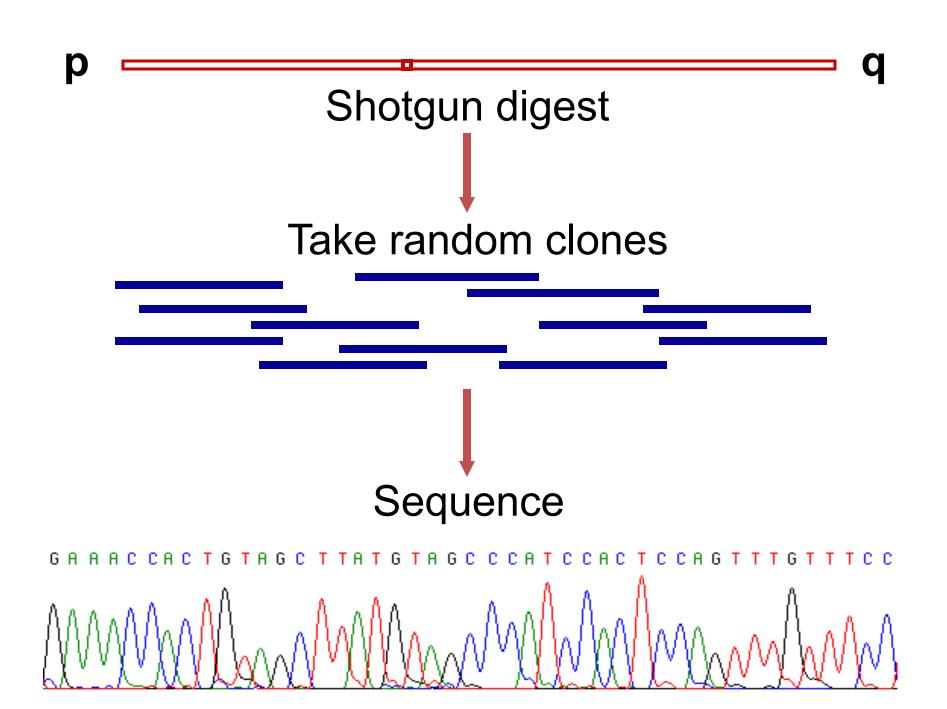


Then came Celera.....

Celera Corp. said that it would sequence the genome faster and cheaper than the public project could.

They used a method known as random shotgun sequencing.

They broke the genome up into 2000 and 10,000 bp pieces, sequenced them, and wrote a computer program to put it all back together.

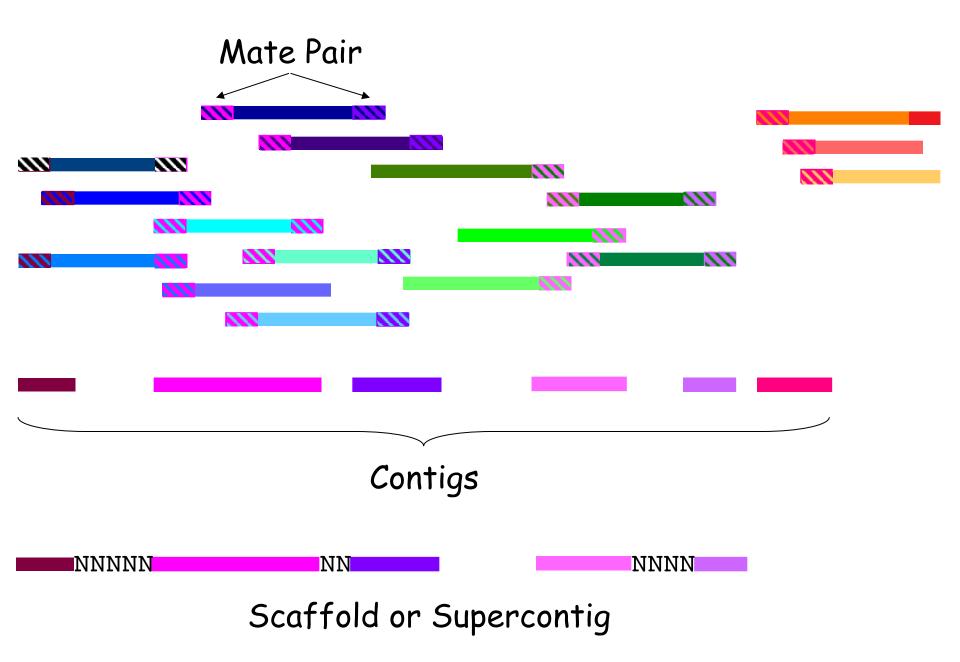


Whole Genome Shotgun

Whole genome shotgun didn't actually sequence whole pieces of DNA, but just their ends.

The WGS reads average ~500bp.

They do however give information in terms of matepairs as to which piece falls where, and that gives a method of ordering and a measure of the size of the holes



It is important to note that not all genome sequences are organized into chromosomes. Those that are being sequenced by whole genome shotgun, where mapping has not taken place, are organized into "linkage groups"

The problems with shotgun...

There are several problems with shotgun sequencing:

- repetitive elements
- gene families
- need more sequence

"x" coverage

- What does 5x coverage of the genome mean?
- That 5 times the number of bases in that genome were sequenced (so for human 5 x 3x10⁹ bases)
- It does NOT mean that the whole genome was covered 5 times

The public reaction....

The public project was concerned that Celera would finish first, and as a commercial company, try to patent significant parts of the genome.

To make the public effort faster, they decided to shotgun the existing BACs.

This lead to HTGs

High Throughput Genome sequence

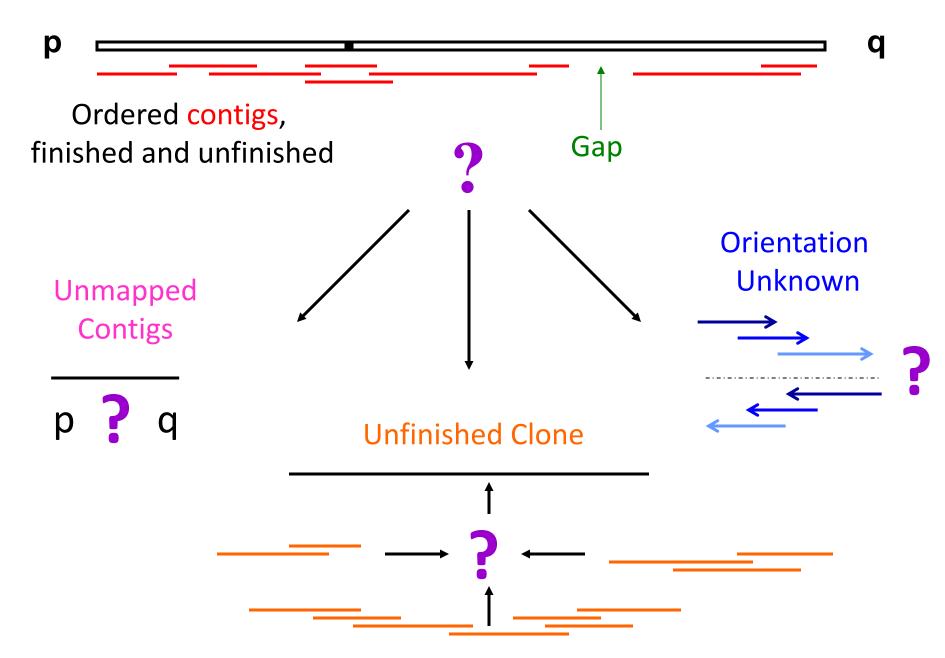
The output of the public project had been well ordered well finished sequence.

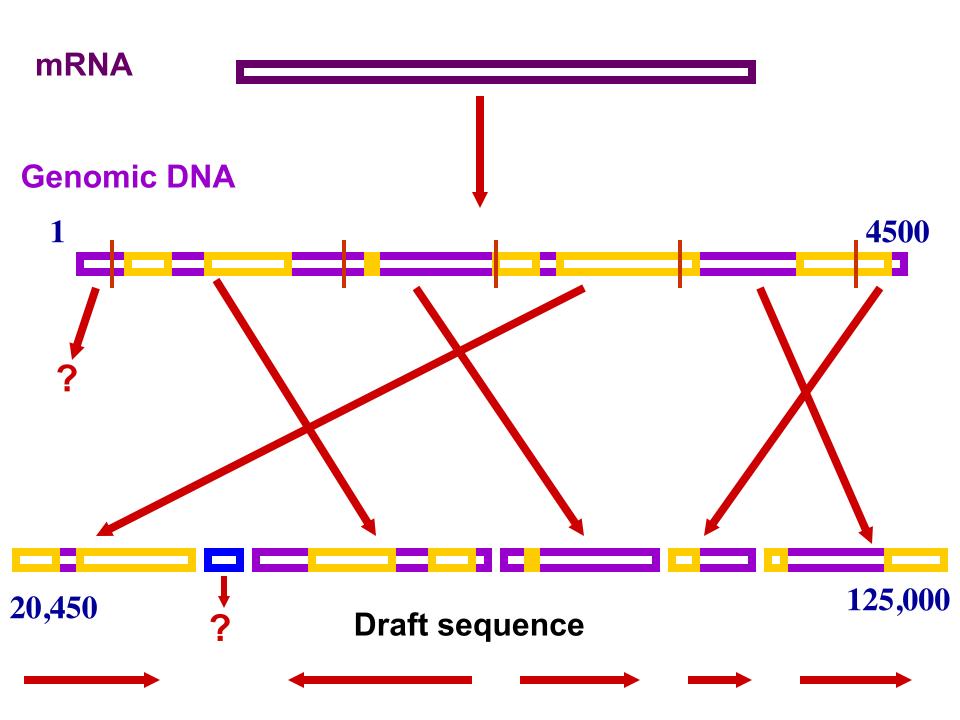
As a result of shotgun sequencing, we ended up with "draft" sequence – sequence whose general location is known, but the exact order or direction of the pieces is not.

HTGS

<u>Status</u>	Location	Definition
Phase 0	HTG division	single-few pass reads of a single clone (not contigs).
Phase 1	HTG division	Unfinished, may be unordered, unoriented contigs, with gaps.
Phase 2	HTG division	Unfinished, ordered, oriented contigs, with or without gaps.
Phase 3	Primary division	Finished, no gaps (with or without annotations).

Draft Sequence - Spring 2000





Sequencing Problems

- Physical Holes (no clones)
- Sequence Holes (no sequence)

Assembly Problems

- Repetitive elements
- Gene families
- Pseudogenes
- Duplication

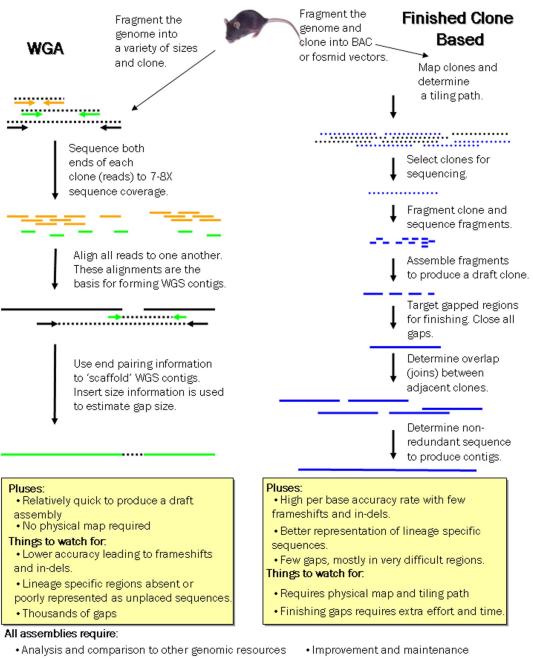


Figure Legend Unsequenced BAC Unsequenced clone from tiling path WGS contig Sequence reads

Genome sequencing with NGS

- NGS is quicker and cheaper than older methods
- Shorter pieces makes the problem of putting everything together worse
- Long read technology can help bridge gaps, and may be the way forward, but still has a high error rate

The post-genomic era.....

Now that we have most of the genome sequence, efforts are being turned to understanding the wealth of information produced:

Defining the genes, when and where they are expressed, what controls them, who they interact with, and how they are mutated, particularly in disease.

Advantages of Genome Sequence

- Previously, it was "one gene, one postdoc"
- Now that we have a better picture of things, we can study systems, and gene interactions
- Previously, it took years to clone and sequence a gene
- Now, all we need is a little bit of sequence,

and we can look up the rest in the genome

Advantages of Genome Sequence

- Previously, after years spent cloning, more time was needed to sequence the surrounding area of the gene, to start looking into regulatory elements
- Now, we have the surrounding sequence and can start looking for the regulatory elements directly

So we started with genomics....

Now we have "Omics":

- Transcriptome
- Proteome
- Regulome.....

And still plenty of work.....

States of Common Genomes

No Eukaryotic Genome is Truly Finished

- Euchromatin/Heterochromatin
- Duplications
- Gaps
- Alternate alleles/regions
- Multiple sources

T2T Consortium

- Telomere to telomere coverage of a human genome
- But:
 - Didn't sequence a normal genome (hydatidiform mole)
 - -No Y chromosome
 - -Only one source
- New challenge: Human Pangenome Reference Consortium

N50

- A common measure for how well put together a genome is
- The mark where 50% of the clones are above or below that length
- N50 human: 67.8 Mb (build 38)
- N50 mouse: 54.5 Mb (build 38)

Y Chromosome

"The Y chromosome in this assembly contains two pseudoautosomal regions (PARs) that were taken from the corresponding regions in the X chromosome and are exact duplicates:

chrY:10001-2649520 and chrY:59034050-59363566 chrX:60001-2699520 and chrX:154931044-155260560

UCSC genome browser, human genome page

Mouse things to remember

- Strains
 - The reference sequence is from C57BI/6J
 - There is sequence available from:
 Balb/c, C3H, NOD, and differences from 15
 strains
- Y chromosome the original reference mouse was female!

http://www.genomesonline.org

Ideas and slides taken from:

Irit Orr Vered Chalifa Caspi **Dolan DNA Learning Center** Human Genome Project at DOE YourGenome - Sanger Center