Genome Sequencing

Principals of a genome project for a species

- Identify the reference line
    - Typically the line has been used historically in genetic research
    - Or, a resource is available for the line
        - Clone library
        - Mutant lines
        - Mapping parent
- Isolate high quality DNA
- Apply some sequencing approach that fits the budget
- Collect DNA
- Assembly the reads into
    - Contigs
    - Scaffolds
- Order scaffolds using a genetic map into pseudochromosomes
- Annotate the genes
- Use the reference genome in research
    - Discover candidate gene(s) that control a phenotype
    - Develop markers to tract important genes/regions of the genome

Approach to the Actual Genome Sequencing

- Fragment the genomic DNA
- Clone those fragments into a cloning vector
- Isolate many clones
- Sequence each clone

Sequencing Techniques Were Well Established

- Sanger was used for the past twenty years
- Helped characterize many different individual genes.
- Previously, the most aggressive efforts
    - Sequenced 40,000 bases around a gene of interest

How is Genomic Sequencing Different???

- The scale of the effort
    - Example
        - Public draft of human genome
            - Hierarchical sequencing
            - Based on 23 billon bases of data
        - Private project (Celera Genomics) draft of human genome
            - Whole genome shotgun sequencing approach
            - Based on 27.2 billon clones
            - 14.8 billion bases

Result:

- Human Genome = 2.91 billion bases

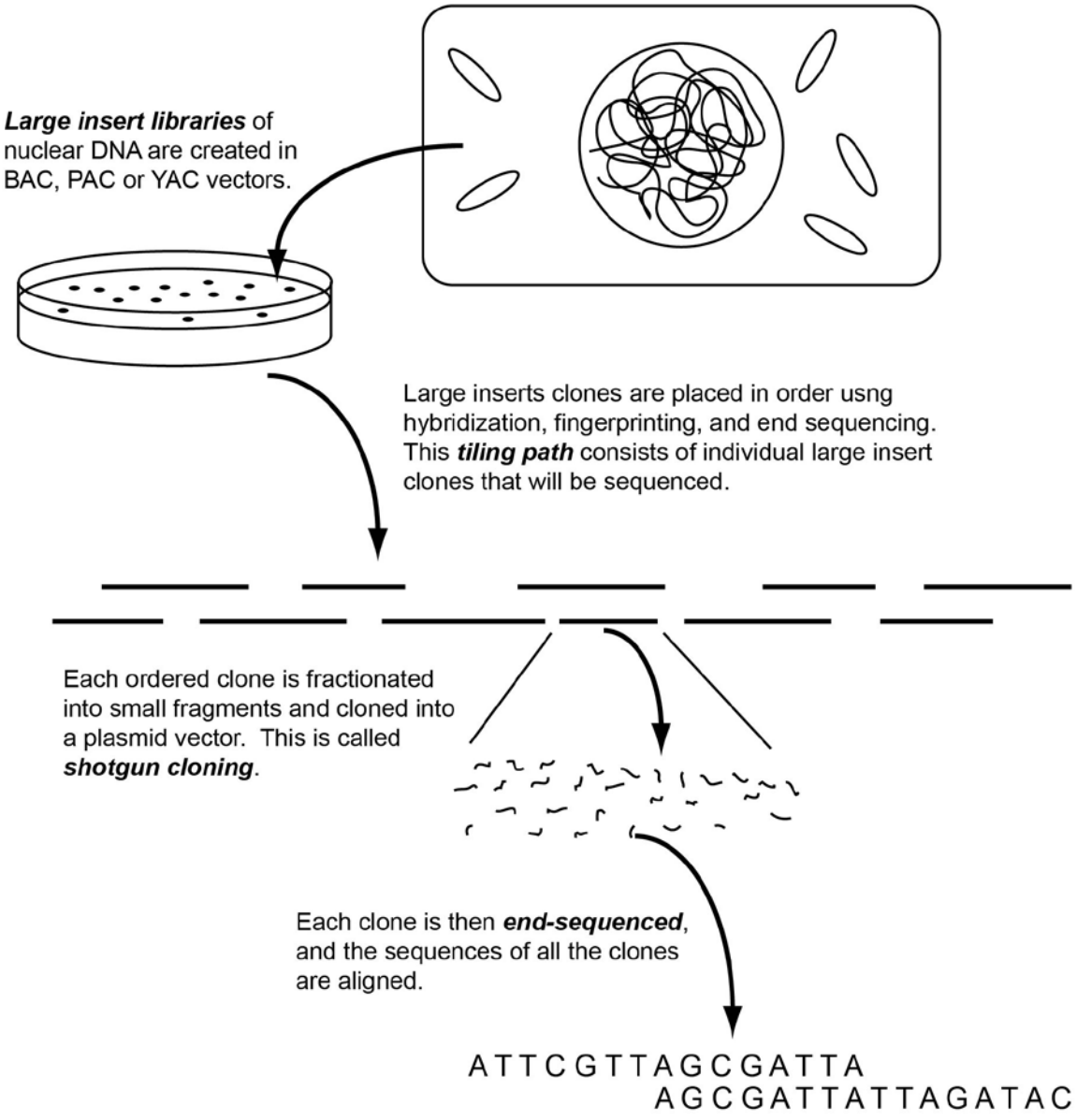
Changes That Facilitated Genomic Sequencing

- Sequencing
  - Basic technique is still the same
- Major changes
  - Thermostable polymerase enzymes
    - Improves quality of sequencing products
  - Fluorescently labeled nucleotides for the reaction
    - Allows for laser detection
  - Laser-based detection systems
    - 8, 16 or 96 samples analyzed simultaneously
  - Results for a single run
    - 500-700 bases of high quality DNA sequence data
  - Human Project peak output
    - 7 million samples per month
    - 1000 bases per second
- Robotics
  - Key addition to genomic sequencing
  - Human hand rarely touches the clone that is being sequenced
  - Robots
    - Pick subclones
    - Distribute clones into reaction plates
    - Create the sequencing reaction
    - Load the plates onto the capillary detection system
  - Result
    - Increased the quality and quantity of the data
    - Decreasing the cost
      - Dropped over 100 fold since 1990
    - Improvements felt in small research lab
  - Sequence reads today
    - $2.50 vs. $15 in the early 1990s.

Hieracrchical Shotgun Sequencing of Genomes

A. The Concept

Hierarchical shotgun sequencing requires that large insert libraries be constructed. A series of these clones are ordered by several techniques. Once these clones are ordered, each clone is separately fractionated into small fragments and cloned into plasmid vectors. The plasmid clones are sequenced, and the sequence is assembled. This is the procedure used to sequence the ***Arabidoposis*** genome, and by the public project to sequence the human genome.



Hierarchical Shotgun Sequencing

- Two major sequencing approaches
  - Hierarchical shotgun sequencing
  - Whole genome shotgun sequencing
- Hierarchical shotgun sequencing
  - Historically
    - First approach
  - Why???
    - Techniques for high-throughput sequencing not developed
    - Sophisticated sequence assembly software not availability
- Concept of the approach
  - Necessary to carefully develop physical map of overlapping clones
    - Clone-based contig (***contig***uous sequence)
  - Assembly of final genomic sequence easier
  - Contig provides fixed sequence reference point
- But
  - Advent of sophisticated software permitted
    - Assembly of a large collection of unordered small, random sequence reads might be possible
  - Lead to **Whole Genome Shotgun** approach

Steps Of Hierarchical Shotgun Sequencing

- Requires large insert library
    - Clone types
        - YAC (yeast artificial chromosomes)
            - Megabases of DNA
            - Few (several thousand) overlapping clones necessary for contig assembly
            - But
                - YACs are difficult to manipulate
                - Most research skilled with bacteria but not yeast culture
            - **Rarely, if ever, used today**
        - BAC or P1 (bacterial artificial chromosomes)
            - Primary advantages
                - Contained reasonable amounts of DNA
                    - about 75-150 kb (100,000 – 200,000) bases
                - Do not undergo rearrangements (like YACs)
                - Could be handled using standard bacterial procedures

Developing The Ordered Array of Clones

- Using a *Molecular Map*
    - DNA markers
    - Aligned in the correct order along a chromosome
    - Genetic terminology
        - Each chromosome is defined as a ***linkage group***
    - Map:
        - Is reference point to begin ordering the clones
        - Provides first look at sequence organization of the genome

Tomato High Density Marker Collection

Chromosome	# Markers
1	363
2	310
3	242
4	238
5	158
6	202
7	191
8	173
9	184
10	160
11	149
12	136

Marker Type

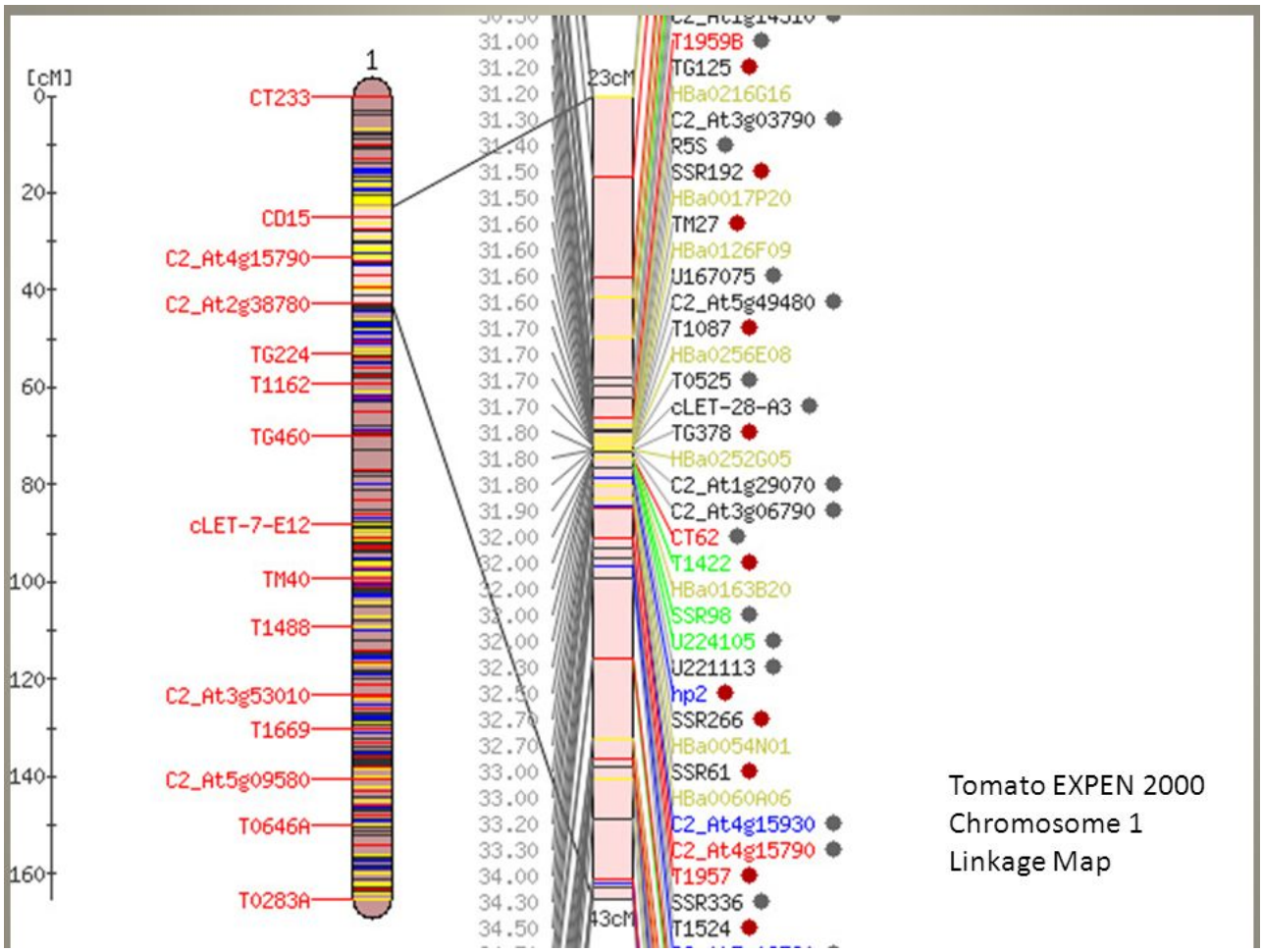
- CAPS (1088), RFLP (1342), SNP (19), SSR (155)

Mapping population

- 88 F2 individuals

Today

- Exclusively SNP markers (n>6,000 SNPs)
- Populations larger (n>200 individuals)



Tomato EXPEN 2000
Chromosome 1
Linkage Map

Developing a Minimal Tiling Path

- Definition
    - Fewest clones necessary to obtain complete sequence
- Caution is needed
    - Clones must be authentic
    - Cannot contain chimeric fragments
        - Fragments ligated together from different (non-contiguous) regions of the genome
    - How to avoid chimeras and select the minimum path
        - Careful fingerprinting

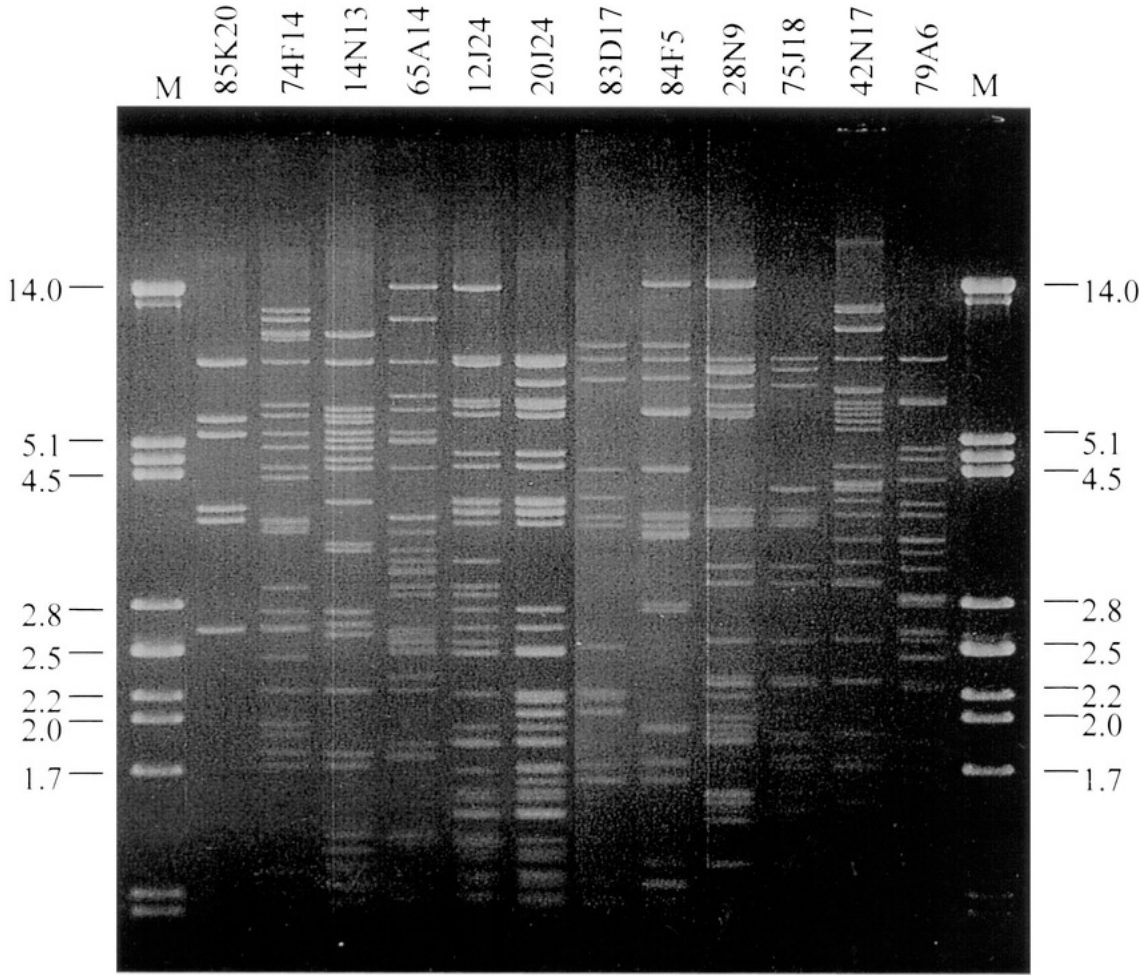
- Overlapping the clones
    - Maps not dense enough to provide overlap
    - *Fingerprinting* clones
        - Cut each with a restriction enzyme (*Hin*dIII)
        - Pattern is generally unique for each clone
        - Overlapping clones defined by
            - Partially share fingerprint fragments
    - Overlapping define the ***physical map*** of the genome

BAC clone fingerprinting

- Restriction enzyme digestion
- Digital, imaging, scoring and aligning

Gel Photograph of digested BAC clones

(https://www.researchgate.net/profile/T_Mirkov/publication/11896126/figure/fig3/AS:394567875612700@1471083720903/Fig-3-HindIII-fingerprinting-gel-of-the-major-BAC-clones-in-the-12-Mb-contig-that.ppm)



Digital Image of Clones

(https://www.researchgate.net/figure/6535067_fig5_Fig-5-Example-of-the-clone-order-fingerprints-of-a-BAC-contig-of-the-apple-physical)

Genomic Physical Maps

- Human
    - 29,298 large insert clones sequenced
        - More than necessary
        - Why???
            - Genomic sequencing began before physical map developed
            - Physical map was suboptimal
- *Arabidopsis*
    - 1,569 large insert clones defined ten contigs
        - Map completed before the onset of sequencing
        - Smaller genome
            - about 125 megabases
- Yeast
    - 493 cosmid (smaller insert clones) clones
        - Relatively high number of clones for genome size

Other Uses Of A Physical Maps

- Rich source of new markers
- Powerful tool to study genetic diversity among species
- Prior to whole genome sequencing
    - Markers can locate a target gene to a specific clone
    - Gene can be sequenced and studied in depth

Sequencing Clones Of The Minimal Tiling Path

- Steps
    - Physically fractionate clone in small pieces
    - Add restriction-site adaptors and clone DNA
        - Allows insertion into cloning vectors
            - Plasmids current choice
    - Sequence data can be collected from both ends of insert
        - ***Read pairs or mate pairs***
            - Sequence data from both ends of insert DNA
            - Simplifies assembly
            - Sequences are known to reside near each other

Assembly of Hierarchical Shotgun Sequence Data

- Process
  - Data collected
  - Analyzed using computer algorithms
  - Overlaps in data looked for
- Accuracy levels
  - Analyzing full shotgun sequence data for a BAC clone
    - Goal: 99.9% accuracy
    - 100 kb BAC clone
      - 2000 sequence reads
      - Equals 8-10x coverage of clone
      - Typical level of accuracy that is sought
    - Primary software used is Phrap
      - **Phrap** = f(**ph**)ragment **a**ssembly **p**rogram
      - Efficient for a "small" number of clones
        - Small relative to number from a whole genome shotgun approach
      - Each sequence read is assesses for quality by the companion software **Phred**
      - Assembles sequence contigs only from high quality reads
  - Working draft sequence
    - 93-95% accuracy
    - 3-5 x coverage of 100 kb BAC clone

Viewing the Quality Score Data

Here the Phred scores are overlaid on the chromatogram of a Sanger sequencing output.

- This is just one format the data can be visualized.
- The visualization comes from a quality score data file generated by base-call machine.

From: http://assets.geneious.com/manual/8.1/GeneiousManualse29.html

Finishing The Sequence

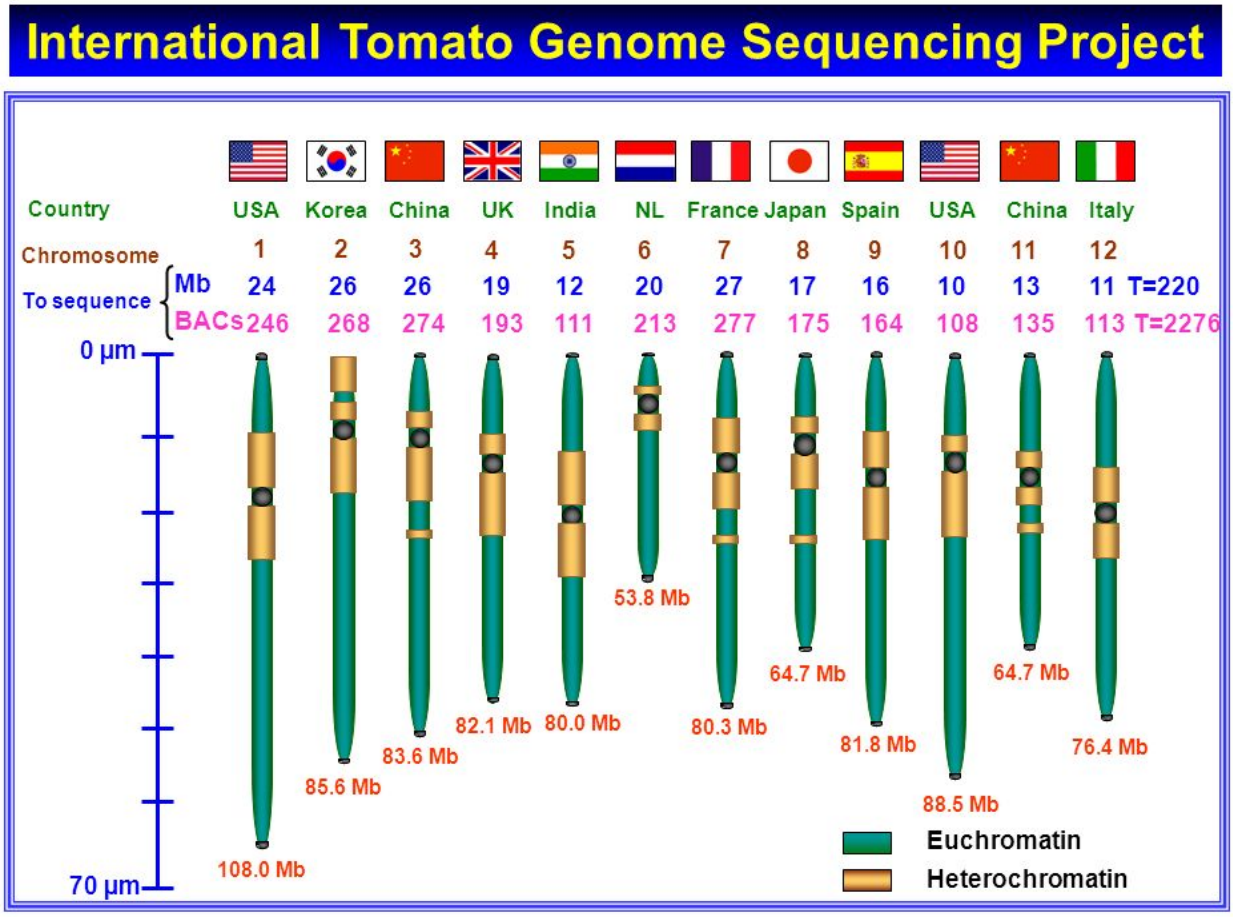
- Gaps need to be filled
    - Study more clones
        - Additional subclones sequenced
        - Tedious and expensive
    - Directed sequencing of clones or genomic DNA used
        - Create primers near gaps
            - Amplify BAC clone DNA
                - Sequence and analyze
            - Amplify genomic DNA
                - Sequence and analyze

Confirming the Sequence

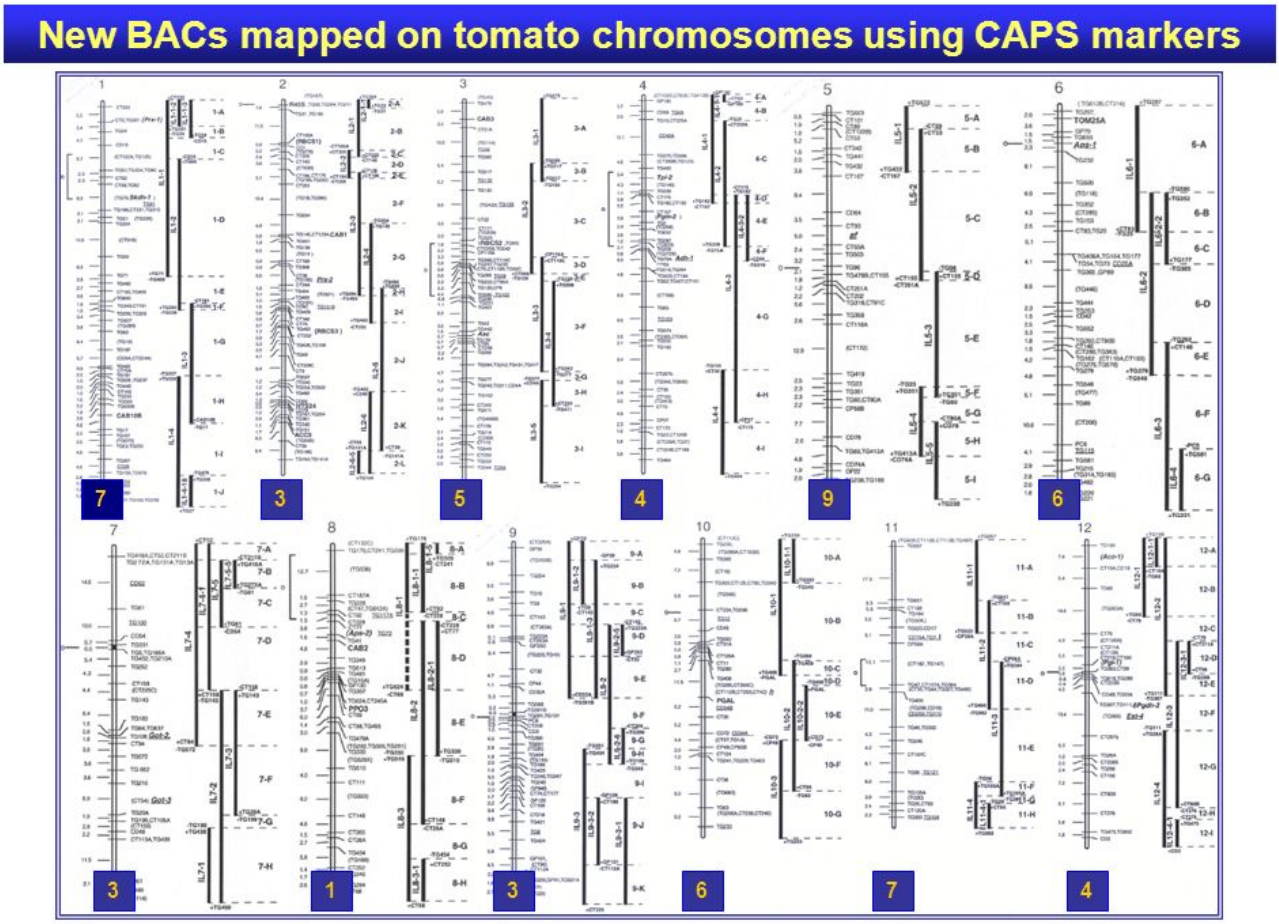
- Molecular map data
    - Molecular markers should be in proper location
- Fingerprint data
    - Fragment sizes should readily recognized in sequence data

Tomato Genome Project

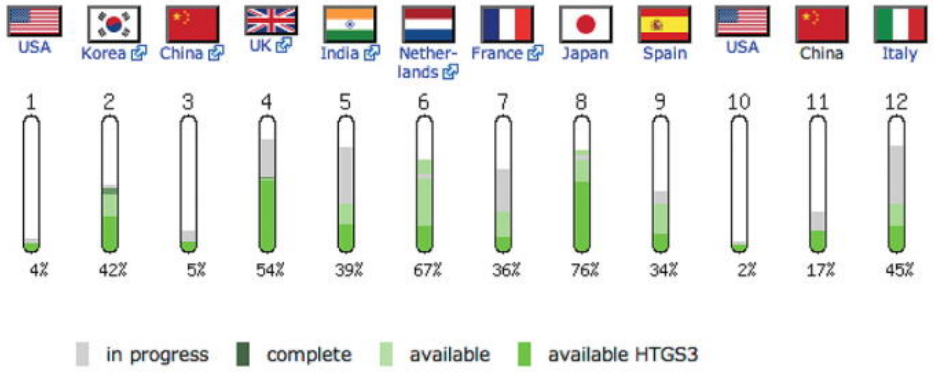
- **Distribution of BAC sequencing effort across the genome**



- **BAC selection for the tomato genome project**



Visualizing the progress in the Tomato Genome Project



BACs													Total
Chr Total	391	268	274	193	111	213	277	175	164	186	135	113	2,500
In progress	8	2	20	54	48	5	92	5	13	1	18	51	317
Complete	11	159	0	87	20	152	53	133	67	4	6	20	712
Available	19	113	15	105	44	144	100	133	57	4	23	51	808
HTGS 1	5	0	0	0	0	116	63	0	13	0	1	21	219
HTGS 2	0	0	0	0	28	0	36	2	30	0	10	11	117
HTGS 3	14	65	15	103	20	36	21	90	18	4	22	19	427
% Done	3%	47%	4%	56%	35%	55%	28%	69%	35%	2%	13%	34%	

Overall Stats

30% of sequencing is complete

29% of BACs are reported finished

32% of BACs have downloadable sequence

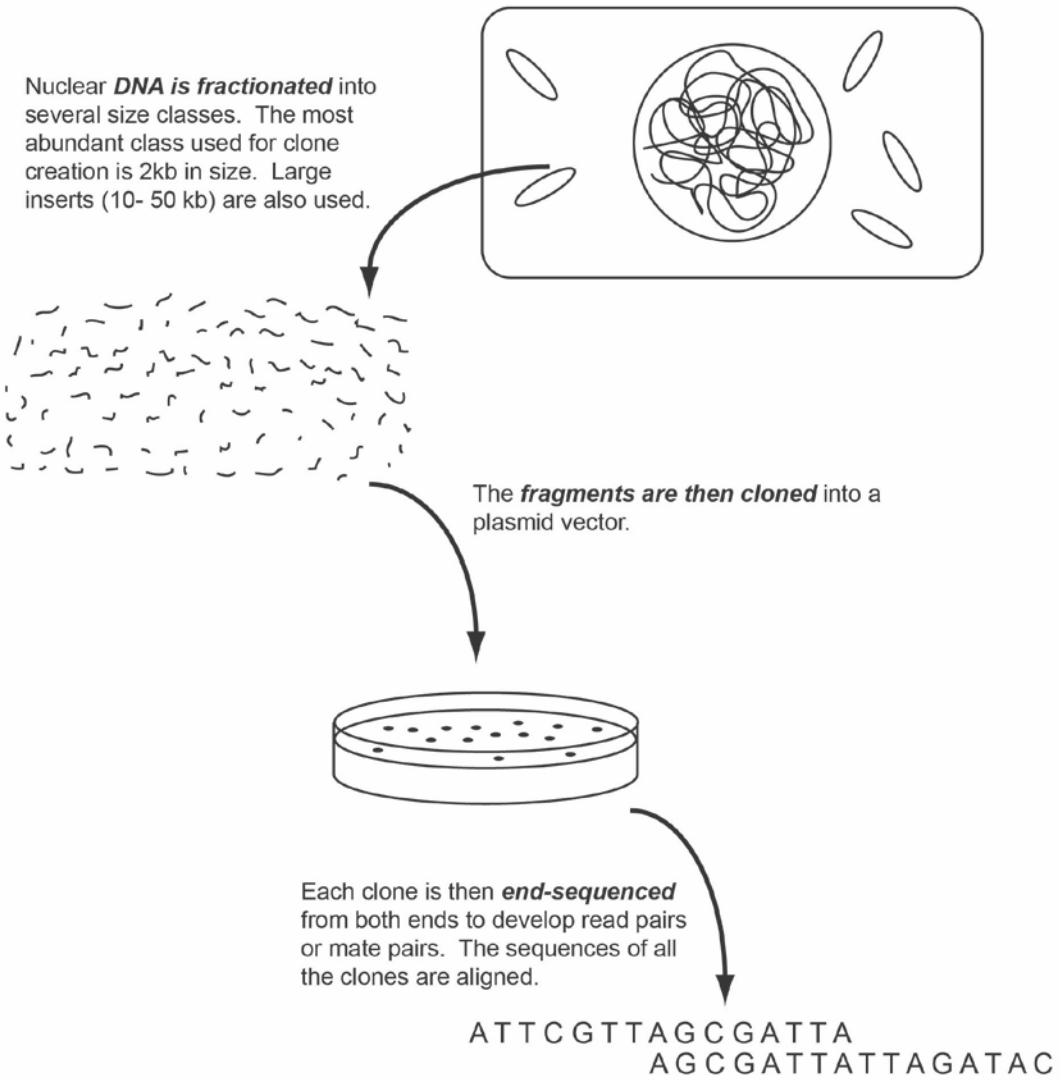
Headlines of Human Genome Sequencing Project

- February 2001
  - Working draft announced
  - Major worldwide news event
- April 2003
  - Finished draft announced
  - Little fanfare
  - Data more useful

Whole Genome Shotgun Sequencing

A. The Concept

Shotgun sequencing requires that random, small insert libraries are created from the total nuclear DNA of the species of interest. A plasmid cloning vector is used for this step. These clones are then sequenced. This step is analogous to the shotgun cloning and sequencing step used for each large-insert clone used in hierarchial shotgun. The sequences of the clones are then aligned. This is the procedure used to sequence the ***Drosophila*** genome, and by Celera to sequence the human genome.



Whole Genome Shotgun Sequencing (WGS)

- Hierarchical sequencing approach
    - Begins with the physical map
    - Overlapping clones are shotgun cloned and sequenced
- WGS
    - Bypasses the mapping step
- Basic approach
    - Take nuclear DNA
    - Shear the DNA
    - Modify DNA by adding restriction site adaptors
    - Clone into plasmids
        - Plasmids are then directly sequenced
        - Approach requires read-pairs
            - Especially true because of the repetitive nature of complex genomes

WGS

- Proven very successful for smaller genomes
    - Essentially the only approach used to sequence smaller genomes like bacteria
- Is WGS useful for large, complex genomes?
    - Initially consider a bold suggestion
    - Large public effort dedicated to hierarchical approach
    - *Drosophila*
        - Sequenced using the WGS approach

- Rice
  - Early publications, not definitive
  - Definitive reference genome developed from hierarchialy shotgun sequencing
  - Two different rice genomes sequenced using WGS approach
    - Only developed a working draft though
    - Public hierarchical sequence available; publication released in August 2005

WGS – Major Challenge 1

- Assembly of repetitive DNA is difficult
  - Retrotransposons (RNA mobile elements)
  - DNA transposons
  - Alu repeats (human)
  - Long and Short Interspersed Repeat (LINE and SINE) elements
  - Microsatellites
- Solution
  - Use sequence data from 2, 10 and 50 kb clones
    - Data from fragments containing different types of sequences can be collected
    - Paired-end reads collected
  - Assembly Process
    - Repeat sequences are initially masked
    - Overlaps of non-repeat sequences detected
    - Contigs overlapped to create supercontigs
  - Software available but is mostly useful to the developers
    - Examples: Celera Assembler, Arcane, Phusion, Atlas

WGS – Major Challenge 2

- For the two sequences approaches
- Assembly is a scale issue
    - WGS approach
        - Gigabytes of sequence data
    - Hierarchical approach
        - Magnitudes less
    - On-going research focuses on developing new algorithms to handle and assembly the huge data sets generated by WGS

Mouse WGS Data

- 29.7 million reads
- 7.4x coverage
- Newer software
- Assembled without mapping or clone data
    - Human WGS had access to this data from the public project
- 225,000 contigs
    - Mean length = 25 kilobases in length
- Super contig subset
    - Mean length =16.9 megabases
- 200 largest supercontigs
    - Anchored using mapping data
    - Represents 96% (9187 Mb) of the euchromatic region of genome

Rat Genome Project: A combined approach

- Nature (2004) 428:493
- Combination of hierarhcial shotgun and whole genome shotgun sequencing

- WGS sequence reads
  - 36 million quality reads (34 million used for assembly)
  - 7X coverage
  - 60%: Whole genome shotgun data
  - Insert size: <10 kb, 10 kb, 50 kb, >150 kb
  - 40% BAC data
  - Small insert clones from the BAC

- BAC Skim
  - A low density sequence analysis of a BAC
  - 21,000 clones analyzed
  - 1.6X coverage

- Enriched BACS
  - Sequences developed by combining WGS data and BAC skim data

- BAC Fingerprinting
  - 200,000 BACs fingerprinted
  - 12X coverage
  - 11,274 fingerprint contigs (FPC) developed
  - Clones selected from contigs for BAC skim

- Bactig
  - Overlapping BACs
  - 1MB in length

- Superbactigs
  - Bactigs joined by paired-end reads
  - Mean = 5MB in length
  - 783 total for the genome

- Ultrabactigs
  - Mean = 18 MB
  - 291 total for genome
  - Synteny data, marker data, and other data used to define the ultrabactig

N50 and L50: Measures of the Quality of Genomes

Contig

- An aligned group of reads that represent one section of the genome
    - No missing sequence data

Scaffolds

- Groups of contings that define a section of the genome
    - Larger than contigs
    - Can contain gaps (missing sequence) that are filled in with Ns
    - Number of scaffolds is always smaller than the number of contigs

Pseudochromosome

- Group of scaffolds that represent one chromosome of the species

N50

- The number of contigs (or scaffolds) whose collective distance equals 50% of the genome length
    - This is a **NUMBER**

L50

- The length of the smallest contig (or scaffolds), of the collection of the contigs (or scaffolds)that comprise the set of N50 contigs (or scaffolds)
    - This is a **LENGTH**

IMPORTANT NOTE

Today, the L50 length is almost always reported as the N5

Short-read Sequencing Projects

The Panda Project (genome = 2.4 Gb)

- **First genome assembled fully from short reads**

Sequencing

- 37 paired-end libraries
  - 150 bp, 500 bp, 2 kb, 5 kb, 10 kb in size
- 176 Gb usable sequence data
  - 73x coverage

Assembly

- SOAPdenovo used for assembly
  - Part of SOAP software package
    - Short Oligonucleotide Analysis Package
      - "SOAPdenovo uses the de Bruijn graph algorithm and applies a stepwise strategy to make it feasible to assemble the panda genome using a supercomputer (32 cores and 512 Gb random access memory (RAM)."
- Poor library and low quality reads excluded
  - 134 Gb sequence data used
    - 56x coverage

Step 1. Contig building

- Data from 500 bp or smaller libraries used first
- Assembly halted when repeat region encountered
    - 39X coverage achieved
    - N50 = 1.5 kb
    - Length = 2.0 Gb

Step 2. Scaffold building

- Paired-end data from all libraries used
    - N50 = 1.3 Mb
    - Total length = 2.3 Gb

Step 3. Closing the gaps

- Local assembly (within a specific gap) using paired end read with one end in a contig and the other in a gap
    - 223.7 Mb gaps closed
    - 54.2 remained unclosed

Step 4. Compare with other carnivores

- Determined that gaps most likely repetitive elements

Genome Assembly

Goal of assembly

- Create contigs based on similarity of sequence reads

Issues that make assembly difficult

- **Sequencing errors**
    - Hard to ascertain, so ignored during assembly
- **Repetitive sequences**
    - Some found 100,000 times
    - Repeats will lead to incorrect assembly
    - Hard to know which sequences overlap
    - Brings two regions together that are not in fact together
    - Resolving some repeats
        - If repeat is shorter than read length, assembly is possible
- **Unclonable sequences**
    - Some sequences lethal to bacteria
        - Cannot be cloned, so sequence data is missing
        - Not an issue with massively parallel sequencing
- **How to overcome these problems**
    - Finishing
    - But finishing is expensive
        - Want to ensure that most of the sequence data available is used in assembly

Result of errors, suboptimum coverage, repeats

- Many more contigs than expected

Assembly problem

- Finding the shortest supersting (T) from a set of strings (s_1,s_2,…s_n)

Features of original assembly algorithms

- Greedy Algorithm Approach
    - Compute all possible overlaps between strings and assign a quality score
    - Merge strings with highest score
    - Continue until no other strings can be merged
    - Uses greedy algorithm
- Fastest method to a solution
- Doesn’t guarantee optimum solution
    - Approach doesn’t work for large genomes
    - Large RAM memory requirements

Next generation assembly algorithms

- Graph theory approach
    - Graph definition
        - A mathematical structure that models pairs of objects from a collection of objects
            - For sequencing the objects are sequence reads
- Overlap-layout consensus approach
    - Set a sequence as a node
    - Overlaps are edges
    - Contig is a path of nodes and edges
- Process
    - Find all possible alignments
    - Remove overlap duplications
    - Construct consensus to create contig

ARACHNE Assembly Program
Genome Research (2002) 12:177; Genome Research (2003) 13:91

Data Preparation

1. Trim low quality sequences at ends of reads
2. Drop entire reads with low overall read scores
3. Trim vector sequences and any known contaminant sequences

Alignment of reads

1. Create table of k-mer (k=24) sequences
    a. each entry associated with a read and position in read
2. High frequency k-mers dropped (repetitive sequences)j
3. Read pairs sharing k-mers identified
4. Overlapping k-mers are merged
5. Shared k-mers extended
6. Alignments refined

Error Correction and Quality Scoring

1. Multiple alignments of overlapping reads created
2. Low frequency errors (20 C vs. 1 T) converted to consensus sequence
3. Insertions/deletions are corrected
4. Quality score attached to alignment

Building the Contig and Repeat Contig

1. Plasmids (of same insert size) containing paired reads from both ends are identified; these are called **paired reads**
2. Paired reads are merged into contigs that do not cross a repeat region
3. Contig built until a repeat boundary is confronted
    a. These are called *unitig* (***uni***que con***tig***)
4. Repeat contig, formed by collapsing identical sequences from unique regions, are marked

a. Repeat contigs have high copy number

b. Repeat contigs are difficult to assembly with other contigs

Supercontigs

1. Unitigs containing two forward and two reverse links are merged
    a. Contigs with the most links and over the shortest distance are preferred
2. Process repeated by merging previously merged contigs into ***supercontigs***
3. Repeat contigs used in an attempt to fill gaps between supercontigs

Arachne2

1. Extended supercontigs
2. Tested for weak and strong supercontigs with misassembly
3. Reassembled these questionable supercontigs

Arachne Whole Genome Assembler

Genome Research 12:177 (2002)

1. Breaks 600 nt read into 24 nt sequences and note read origin of the sequence
2. Create database with each sequence as main entry
    - Each sequence entry contains frequency and read identifier data
4. Discard high copy reads (these are repeats)
5. Align reads from low frequency sequences
6. Discover mate pairs represented in two plasmids of same length
    - These are paired pairs
7. Find a mate pair that matches only one end of the paired pair
    - Sequences are considered to be a single large read
8. Process continues until a repeat is encountered
9. Assembly stops and a unique contig is declared
10. Overlaps of unique contigs discovered
11. Supercontigs are declared

Common Bean: 454-based Project

Sequencing Libraries

Library	Sequencing Platform	Average Read/Insert Size	Read Number	Assembled Sequence Coverage
Linear	454 XLR & FLX+	362	38,107,155	18.64x
GPNB	454 XLR paired	2,798 ± 1,047	589,346	0.11x
GGAS	454 XLR paired	3,922 ± 643	1,940,576	0.41x
GXSF	454 XLR paired	3,991 ± 337	467,414	0.07x
HYFA	454 XLR paired	4,729 ± 497	1,648,022	0.25x
HYFC	454 XLR paired	4,736 ± 504	1,491,648	0.24x
HYFB	454 XLR paired	4,759 ± 528	1,196,104	0.17x
HXTI	454 XLR paired	8,022 ± 1,016	1,364,808	0.22x
GXNX	454 XLR paired	9,192 ± 1,058	878,832	0.16x
HXWF	454 XLR paired	11,903 ± 1,928	724,196	0.13x
HXWH	454 XLR paired	12,231 ± 1,902	413,396	0.08x
VUK (Fosmid-end)	Sanger	34,956 ± 4,536	240,384	0.20x
VUL (Fosmid-end)	Sanger	36,001 ± 4,632	88,320	0.08x
PVC (BAC-end)	Sanger	121,960 ± 16,572	81,408	0.08x
PVA (BAC-end)	Sanger	126,959 ± 25,658	89,017	0.09x
PVB (BAC-end)	Sanger	135,292 ± 21,487	92,160	0.09x
Total		N/A	49,412,786	21.02x

Genome Assembly Statistics

Comparison of Two Legume Species and Sanger and Short Read Sequence Data Collection

Statistic	Soybean	Common Bean
Sequencing method	WGS, Sanger	WGS, 454 & Illumina
Genome size (contig)	955 Mb (1.9% gap)	473 Mb (9.3% gap)
Genome size (scaffold)	973 Mb	521 Mb
Contig number	16,311	41,391
Contig N50	1,492	3.273
Contig L50	189 kb	39.5 kb
Scaffold number	1,168	708
Scaffold N50	10	5
Scaffold L50	47.8 Mb	50.4 Mb
Genetic map loci	1.536 SNPs	7,015 SNPs

Genome Assembly Statistics

Comparison of Two Sequencing Methods for One Species

Statistic	Common Bean	Common Bean
Sequencing method	WGS, 454 & Illumina	WGS, PacBio
Genome size (contig)	473 Mb (9.3% gap)	532 Mb (1.1% gap)
Genome size (scaffold)	521 Mb	537 Mb
Contig number	41,391	1,044
Contig N50	3,273	73
Contig L50	39.5 kb	1.9 Mb
Scaffold number	708	478
Scaffold N50	5	5
Scaffold L50	50.4 Mb	49.7 Mb
Genetic map loci	7,015 SNPs	7,015 SNPs
% genome in scaffolds >50 kb		99.1%

PacBio Scaffold Sets

Set	Scaffolds	Size
Main genome	478	537 Mb
Mitochondrion	7	448 Kb
Chloroplast	29	662 Kb
Unanchored rDNA	7	296 Kb
Alternative haplotypes	442	9 Mb
Repeat scaffolds	275	10 Mb
Excluded (<1kb)	5	4 Kb

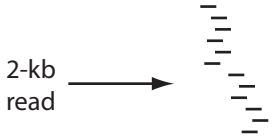
Comparison of Plant Genome Species

Species (Sequencing method)	N50	L50
Brachypodium (Sanger)		
Contigs	252	347.8 Kb
Scaffolds	3	9.3 Mb
Sorghum (Sanger)		
Contigs	958	195.4 Kb
Scaffolds	6	62.4 Mb
Soybean (Sanger)		
Contigs	1,492	189.4 Kb
Scaffolds	10	47.8 Mb
Common Bean (454)		
Contigs	3,273	39.5 Kb
Scaffolds	5	50.4 Mb
Canola (454/Sanger/Illumina)		
Contigs		38.9 kb
Scaffolds		763.7 Kb

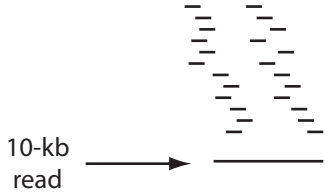
Scaffold Assembly

Building a Scaffold Using Paired-end Reads of Different Sized Sequences

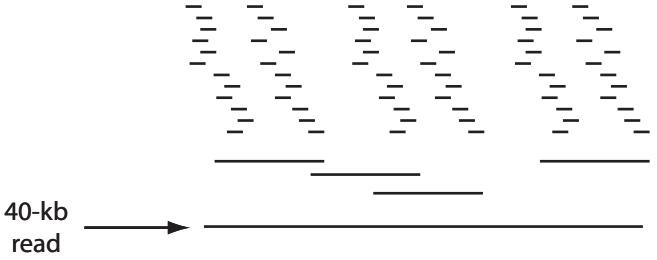
Step 1: Build a contig with overlapping 2-kb paired-end reads



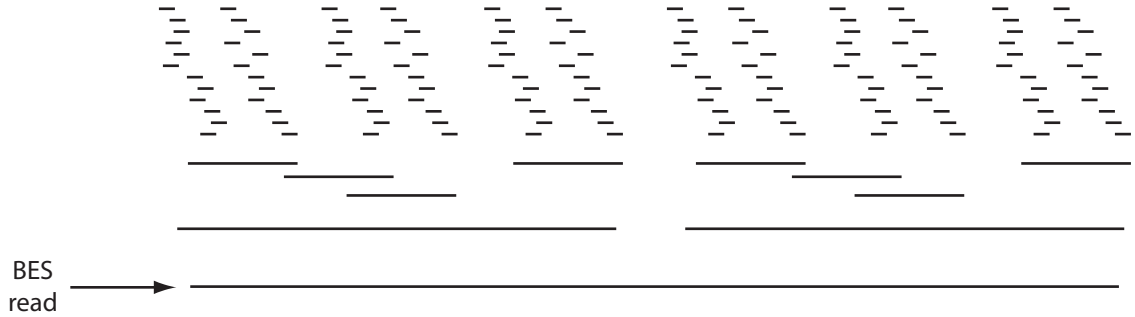
Step 2: Link two contigs with 10-kb paired-end reads



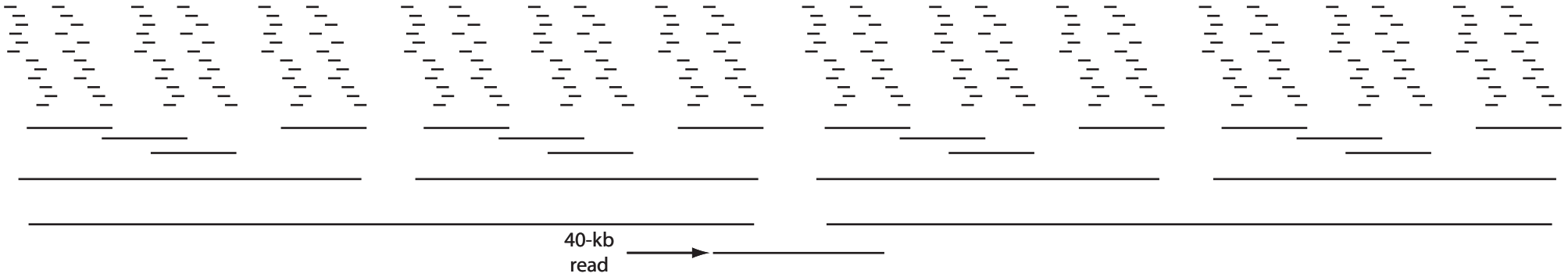
Step 3: Link three 10-kb contigs with 40-kb paired-end reads



Step 4: Link two 40-kb contigs with 100-kb BAC end sequences (BES)



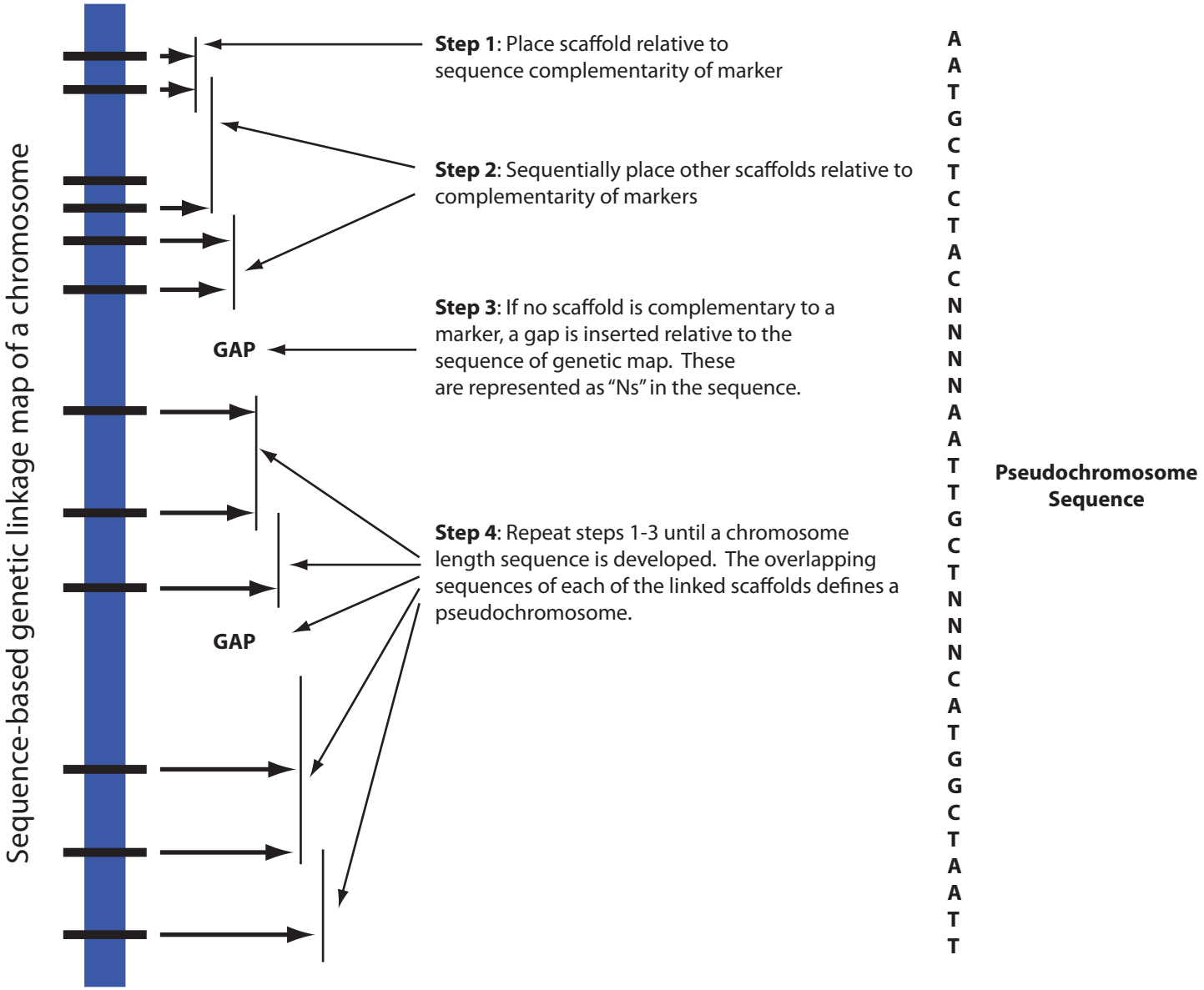
Step 5: Here link two100-kb BAC sized contigs with a 40-kb paired-end read; other sized reads can also be used for this linking



Step 6: Continue linking larger blocks of sequences until the block can not be linked with another block. This block is defined as a scaffold.

Genome Assembly

Linking Scaffolds to a Dense Genetic Map



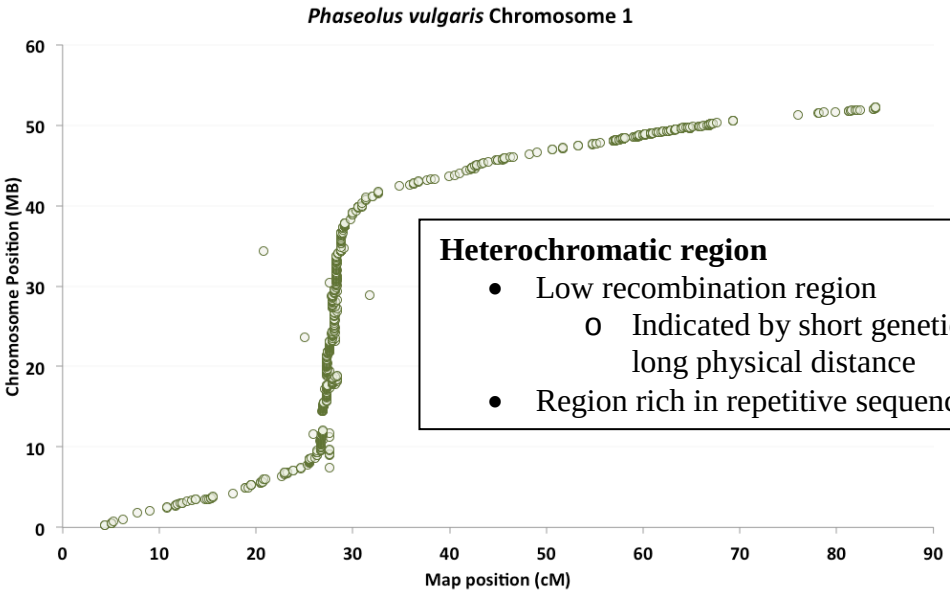
Species name	Common name	Genotype	Year	Publication	Technical method	# Chrom	Est. genome size/assembled size (Mb)	Repeat content (%)	Chrom size range (Mb)	# genes/ transcripts	Contig N50/L50 (#/kb)	Scaffold N50/L50 (#/kb)	Genome duplication history
Arabidopsis thaliana	Arabidopsis	Columbia	2000	Nature 408:796	HSS/S	5	125/135	20[1]	18-29	27,416/ 35,386			Eudicot 3x + Brassicaceae (2x+2x)
Oryza sativa	Rice	Nipponbare	2005	Nature 436:793	HSS/S	12	430/371	45[1]	23-43	39,049/ 49,061			Poales (2x+2x)
Populus trichocarpa	Poplar	Nisqually 1	2006	Science 313:1596	WGS/S	19	485/423	40[1]	11-36	41,335/ 73,013	??/126	??/3,100	Eudicot 3x + (2x)
Vitus vinifera	Grape	PN40024	2007	Nature 449:463	WGS/S	19	475/487	22[1]	10-22	/ 26,346	??/126	??/2,065	Eudicot 3x
Carica papaya	Papaya	Sunup	2008	Nature 452:991	WGS/S	9	372/370	52		27,332/ 27,996	??/11	??/1,000	Eudicot 3x
Sorghum bicolor	Sorghum	BTx623	2009	Nature 457:551	WGS/S	10	818/727[2]	63[1]	50-70	33,032/ 39,441	958/195	6/62,400	Poales (2x+2x)
Zea mays	Maize	B73	2009	Science 326:1112	HSS/S	10	/3,234	84	150-301	39,475/ 137,208			Poales (2x+2x) + (2x)
Cucumis sativus	Cucumber	9930	2009	Nat Genet 41:1275	WGS/S,I	7	??/244	22[1]		21,491/ 32,528	??/227	??/1,140	Eudicot 3x
Glycine max	Soybean	Williams 82	2010	Nature 463:178	WGS/S	20	1115/978	57	37-62	56,044/ 88,647	1,492/189	10/47,800	Eudicot 3x + Legume 2x + (2x)
B. distachyon	Brachypodium	Bd21	2010	Nature 463:763	WGS/S	5	272/275	28	25-75	26,552/ 31,029	252/348	3/59,300	Poales (2x+2x)
Ricinus communis	Castor bean	Hale	2010	Nat Biotech 28:951	WGS/S, 454	10	320/326	~50		31,237/??	??/21	??/497	Eudicot 3x
Malus x domestica	Apple	Golden Delcious	2010	Nat Genet 42:833	WGS/S	17	742/604	36	21-47	63,538/ 63,541	16,171/13	102/1,542	Eudicot 3x + Rosaceae 2x
Jatropha curcas	Jatropha		2010	DNA Res 18:65	WGS/S		380/285	37		40,929/??	??/4		
Theobroma cacao	Cocao	B97-61/B2	2011	Nat Genet 43:101	WGS/S, 454, I	10	430/362	24	12-31	29,452/ 44,405		??/5,624	Eudicot 3x
Fragaria vesca	Strawberry	H4x4	2011	Nat Genet 43:109	WGS/S, 454, I, So	7	240/220	23		32,831/??		??/1,300	Eudicot 3x
Arabidopsis lyrata	Lyrata	MN47	2011	Nat Genet 43:476	WGS/S	8	??/207	30	19-33	32,670/??	1,309/5,200		Eudicot 3x + Brassicaceae (2x+2x)
Phoenix dactylifera	Date palm	Khalas	2011	Nat Biotech 29:521	WGS/I	18	658/381	29		28,890/??	??/6	??/30	
Solanum tuberosum	Potato	DM1-3 516 R44	2011	Nature 475:189	WGS/S, 454, I, So	12	844/727	62		35,119/ 51,472	6,446/31	121/1,782	Eudicot 3x + Solanaceae 3x
Thellungiella parvula	Thellungiella		2011	Nat Genet 43:913	WGS/454, I	7	160/137	8		30,419/??		8/5,290	
Cucumis sativus	Cucumber	B10	2011	PLoS ONE 6:e22728	WGS/S, 454	7	??/323			26,587/??	??/23	??/323	Eudicot 3x
Brassica rapa	Cappage	Chiifu-401-42	2011	Nat Genet 43:1035	WGS/I	10	??/283	40		41,174/??	2,778/27	39/1,971	Brassicaceae 2x + (2x)
Cajanus cajan	Pigeon pea	ICPL 87119		Nat Biotech 30:83	WGS/S, I	11	808/606	52	10-48	40,071	7815/23	380/516	Eudicot 3x + Legume 2x
Medicago truncatula	Medicago		2011	Nature 480:520	WGS/S, 454, I	8	454/384		35-57	44,135/ 45,888		53/1270	Eudicot 3x + Legume 2x
Setaria italica	Foxtail millet	Yugu 1	2012	Nat Biotech 30:555	WGS/S	9	451/406	40	24-48	35,471/ 40,599	982/126	4/47,300	

Species name	Common name	Genotype	Year	Publication	Technical method	# Chrom	Est. genome size/assembled size (Mb)	Repeat content (%)	Chrom size range (Mb)	# genes/ transcripts	Contig N50/L50 (#/kb)	Scaffold N50/L50 (#/kb)	Genome duplication history
Solanum lycopersicon	Tomato	Heinz 1706	2012	Nature 485:635	WGS/S,So	12	900/760	63	45-65	34,727/??			Eudicot 3x + Solanaceae 3x
Linum usitatissimum	Flax	CDC Bethune	2012	Pl Journal 72:461	WGS/I	15	373/318	24		43,484	4,427/20	132/693	Eudicot 3x + (2x)
Musa acuminata	Banana	DH-Pahang, ITC1511	2012	Nature 488:213	WGS/S, 454, I	11	??/523	44	22-35	36,542	/43	/1,311	Zingiberales 2x + (2x + 2x)
Gossypium raimondii	Cotton (B genome diploid)		2012	Nat Genet 44:1098	WGS/I	13	775/567	57	25-69	40,976/??	4,918/45	2,284/95	Eudicot 3x + Gossypium 2x
Azadirachta indica	Neem	Local tree	2012	BMC Genomics 13:464	WGS/I		??/364	13		20,169/??	??/0.7	??/452	
Gossypium raimondii	Cotton (D genome diploid)		2012	Nature 492:423	WGS/S, 454, I	13	880/738	61	35-70	37,505/ 77,267	1596/136	6/62,200	Eudicot 3x + Gossypium 2x
Prunus mume	Chinese plum	2 genotypes	2012	Nature Communications 3:1318	WGS/I	8	??/237	45		31,390/??	2009/32	120/578	
Pyrus bretschneideri	Pear		2013	Genome Research	HSS+WGS/I	17	528/512	53	11-43	42,812/??	??/36	??698	Eudicot 3x + Rosaceae 2x
Cirtullus lanatus	Watermelon	97103	2013	Nat Genet 45:51	WGS/I	11	425/354	45	24-34	24,828/??	??/26	??/2380	Eudicot 3x
Morus notabilis	Mulberry		2013	Nature Communications 4:2445	WGS/I	7	357/330	47		29,338/??	2,638/34	245/390	Eudicot 3x
Phaseolus vulgaris	Common bean	G19833	2014	Nat Genet (in press)	WGS/S, 454,I	11	587/521	45	32-60	27,197/ 31,688	3,273/40	5/50	Eudicot 3x + Legume 2x

Physical Distance vs. Genetic Distance

Typical Chromosome

- High recombination on ends of chromosome
- Low recombination in the center (heterochromatic) region of the genome



Heterochromatic region

- Low recombination region
  - Indicated by short genetic distance and long physical distance
- Region rich in repetitive sequences

Acrocentric Chromosome

- Heterochromatic repeat rich region at end of chromosome