Bioinformatics

CS300

Genome Sequencing and Assembly

Fall 2017

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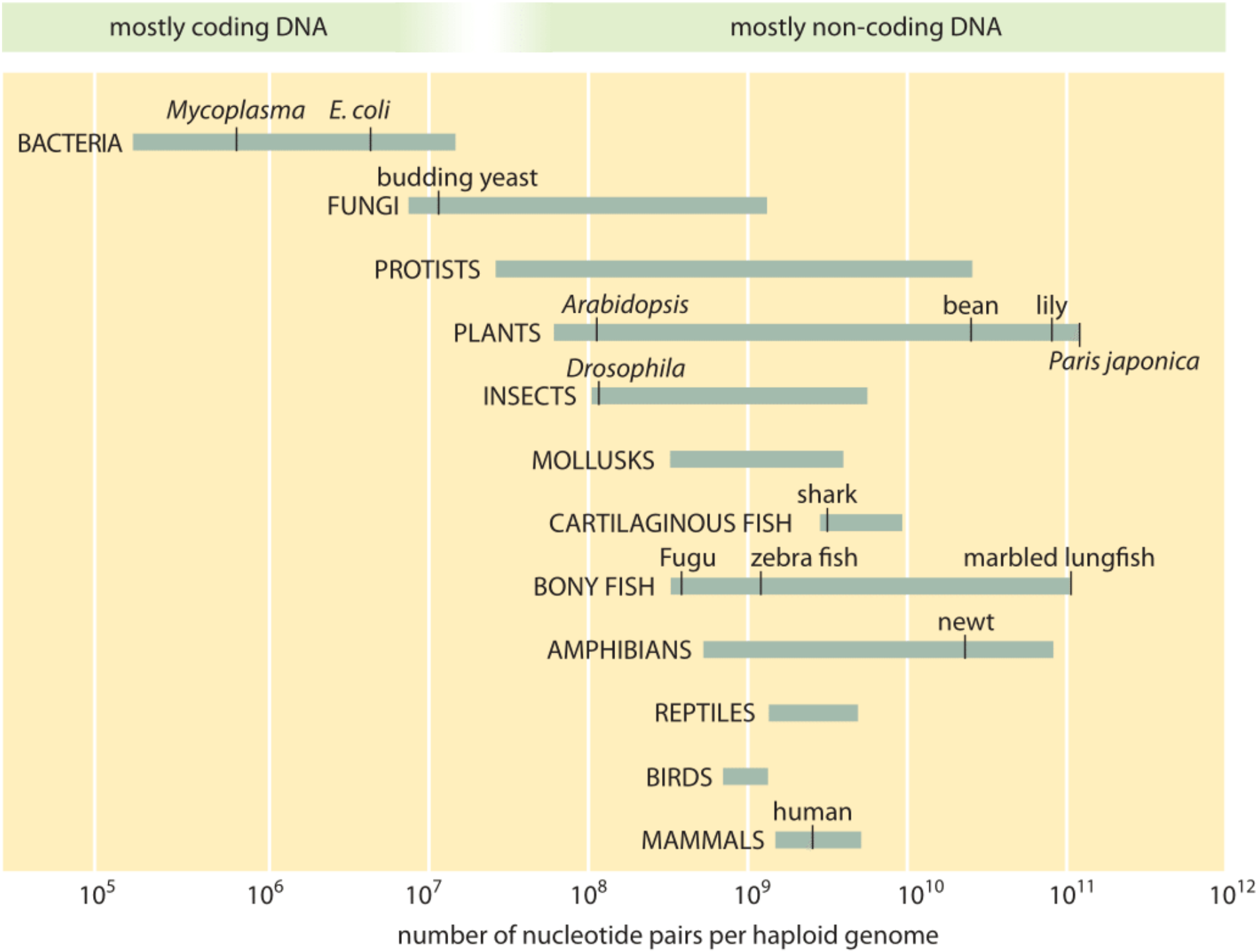
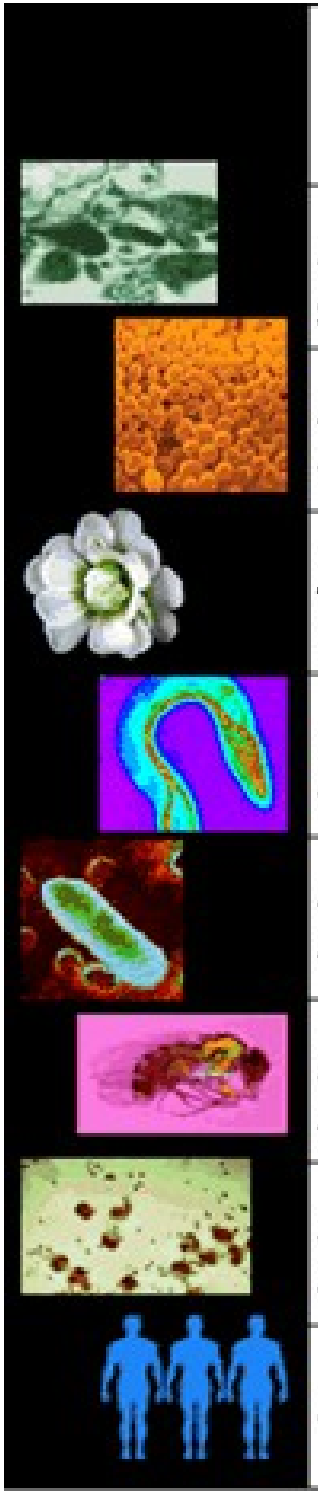
What is a Genome?

- An organism's complete set of DNA, including all of its genes, regulatory regions, non-coding regions, etc.
- An organism's complete set of genetic instructions


mostly coding DNA
mostly non-coding DNA
Mycoplasma
E. coli
BACTERIA
budding yeast
FUNGI
PROTISTS
Arabidopsis
bean
lily
PLANTS
Paris japonica
Drosophila
INSECTS
MOLLUSKS
shark
CARTILAGINOUS FISH
Fugu
zebra fish
marbled lungfish
BONY FISH
newt
AMPHIBIANS
REPTILES
BIRDS
human
MAMMALS
10^5
10^6
10^7
10^8
10^9
10^{10}
10^{11}
10^{12}
number of nucleotide pairs per haploid genome

What Is In a Genome?



Organism	Number of genes in the genome
Myscoplasma genitalium	517
Saccharomyces cerevisiae	6,275
Arabidopsis thaliana	~ 20,000
Caenorhabditis elegans	19,099
Haemophilus influenzae	1,743
Drosophila melanogaster	13,601
Neisseria meningitdis	2,158
Homo sapiens	20,000–25,000

Genome Projects

- Goals:
  - Determine complete genome sequence of an organism
  - Annotate protein-coding genes and other important genome-encoded features

Genome Projects

- Projects:
  - Over 15,000 genome projects in progress or completed

Genome Information by organism

Search by organism | Clear

Download Reports from FTP site

Overview [30649] | Eukaryotes [4874] | Prokaryotes [118997] | Viruses [7497] | Plasmids [10401] | Organelles [10835]

Download selected records

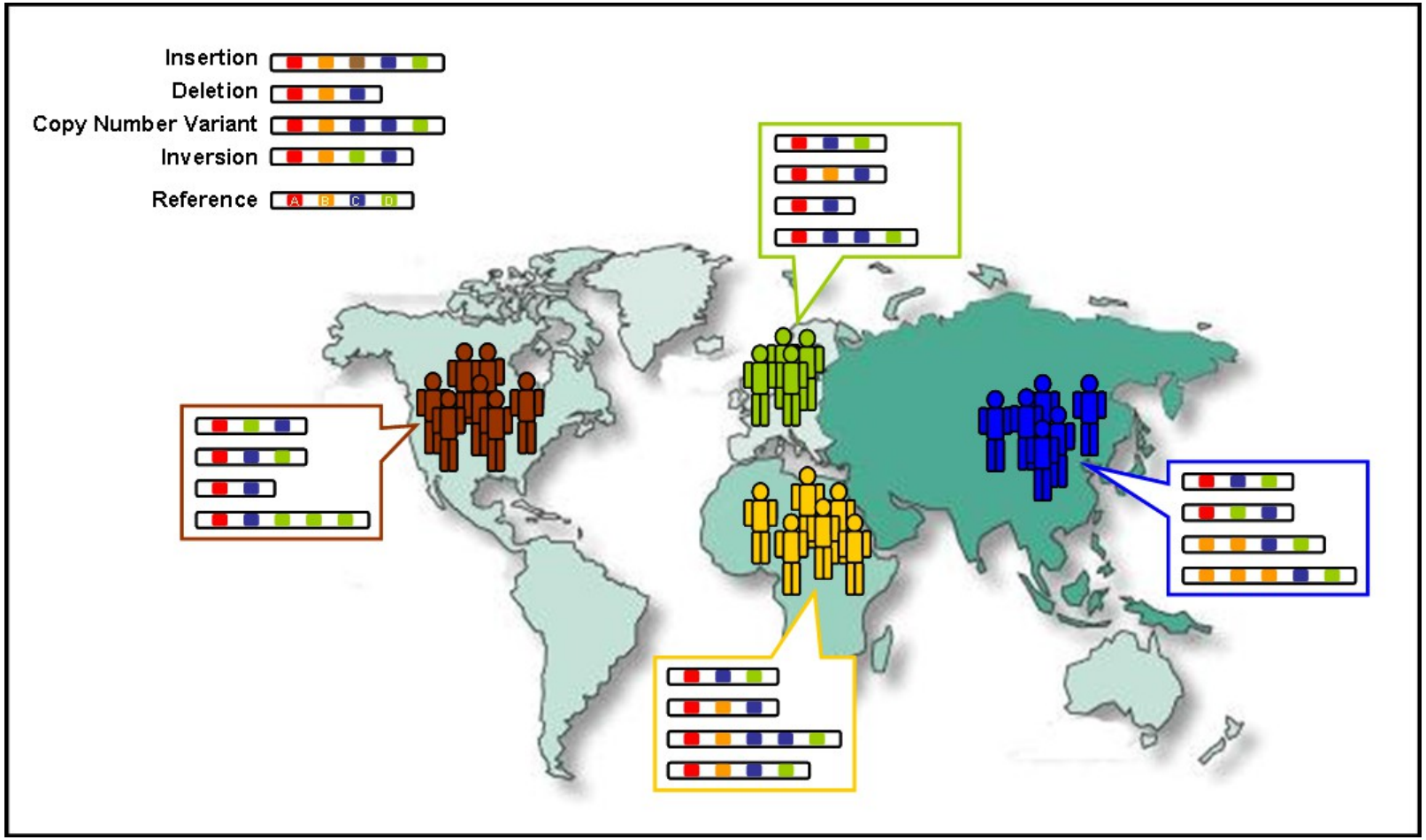
Items 1 - 100 of 30649 << First < Prev Page 1 of 307 Next > Last >>

Organism/Name	Kingdom	Group	SubGroup	Size (Mb)	Chr	Organelles	Plasmids	Assemblies
	All	All	All					
'Chrysanthemum coronarium' phytoplasma	Bacteria	Terrabacteria group	Tenericutes	0.739592	-	-	-	1
'Echinacea purpurea' witches'-broom phytoplasma	Bacteria	Terrabacteria group	Tenericutes	0.545427	-	-	-	1
'Osedax' symbiont bacterium Rs2_46_30_T18	Bacteria	unclassified Bacteria	unclassified Bacteria (miscellaneous)	4.02183	-	-	-	1
Abaca bunchy top virus	Viruses	ssDNA viruses	Nanoviridae	0.006422	6	-	-	1
Abalone herpesvirus Victoria/AUS/2009	Viruses	dsDNA viruses, no RNA stage	unclassified	0.211518	1	-	-	1
Abalone shriveling syndrome-associated virus	Viruses	dsDNA viruses, no RNA stage	unclassified	0.034952	1	-	-	1
Abelson murine leukemia virus	Viruses	Retro-transcribing viruses	Retroviridae	0.005894	1	-	-	1

https://www.ncbi.nlm.nih.gov/genome/browse/

Genome Projects

- Contrast genetic material of populations to determine ancestry



https://en.wikipedia.org/wiki/1000_Genomes_Project#Human_genetic_variation

Genome Projects: Data

- Locate genes for proteins in sequences.

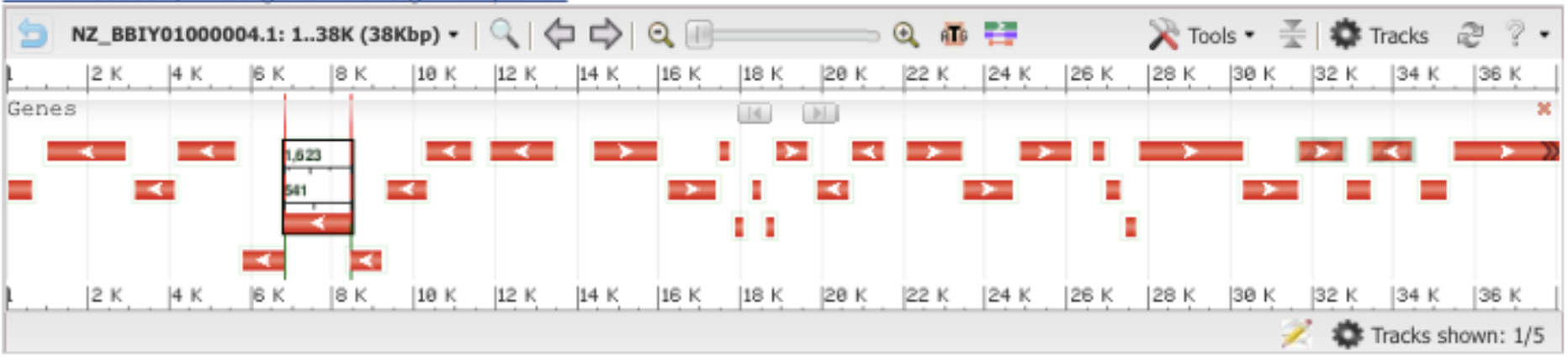
Genome Assembly Annotation

Type	Name	RefSeq	INSDC	Size (Mb)	GC%	Protein	tRNA	Other RNA	Gene	Pseudogene
	master WGS	NZ_BBIY00000000.1	BBIY00000000.1	0.74	27.6	901	27	-	928	-

Genome Region

'Chrysanthemum coronarium' phytoplasma strain OY-V BBIY01000004, whole genome shotgun sequence

Go to nucleotide: Graphics FASTA GenBank



https://www.ncbi.nlm.nih.gov/genome/browse/

Genome Projects: Data

- Protein meta data

'Chrysanthemum coronarium' phytoplasma strain OY-V BBIY01000004, whole genome shotgun sequence

Go to nucleoti

NZ_BBIY01000004.1: 1..38K (38Kbp)

Genes

WP_042067579.1

CDS: WP_042067579.1
Title: sugar ABC transporter substrate-binding protein
Location: complement(6,823..8,445)
[*Length*]
Span: 1,623
Product: 540
[*Qualifiers*]
inference: COORDINATES: similar to AA sequence:RefSeq:WP_011161091.1

Download: WP_042067579.1

Links & Tools
BLAST Genomic: NZ_BBIY01000004.1 (6,823..8,445)
BLAST Protein: WP_042067579.1
BLINK Results: WP_042067579.1
FASTA View: NZ_BBIY01000004.1 (6,823..8,445), WP_042067579.1
GenBank View: NZ_BBIY01000004.1 (6,823..8,445), WP_042067579.1
Graphical View: WP_042067579.1

Run Blast

u are here: NCBI > Genomes & Maps >

TTING STARTED
BI Education
BI Help Manual
BI Handbook
ining & Tutorials
bmit Data

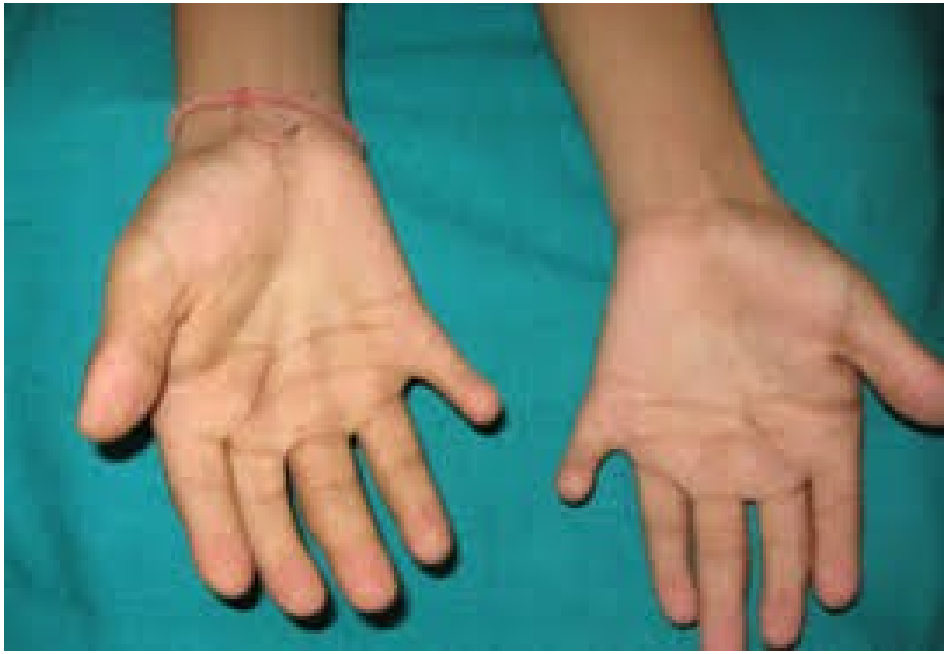
Human Genetic Variation

- Having diverse human genetic information helps to spot genetic conditions
- Genetic drift: a random fluctuation in the population frequency of a trait
  - Occurring in subsequent generations and would result in the loss of all variation in the absence of external influence

Detection By Comparison

- Early detection of genetic problems by being able to compare genomes to some “wild-type” genome.

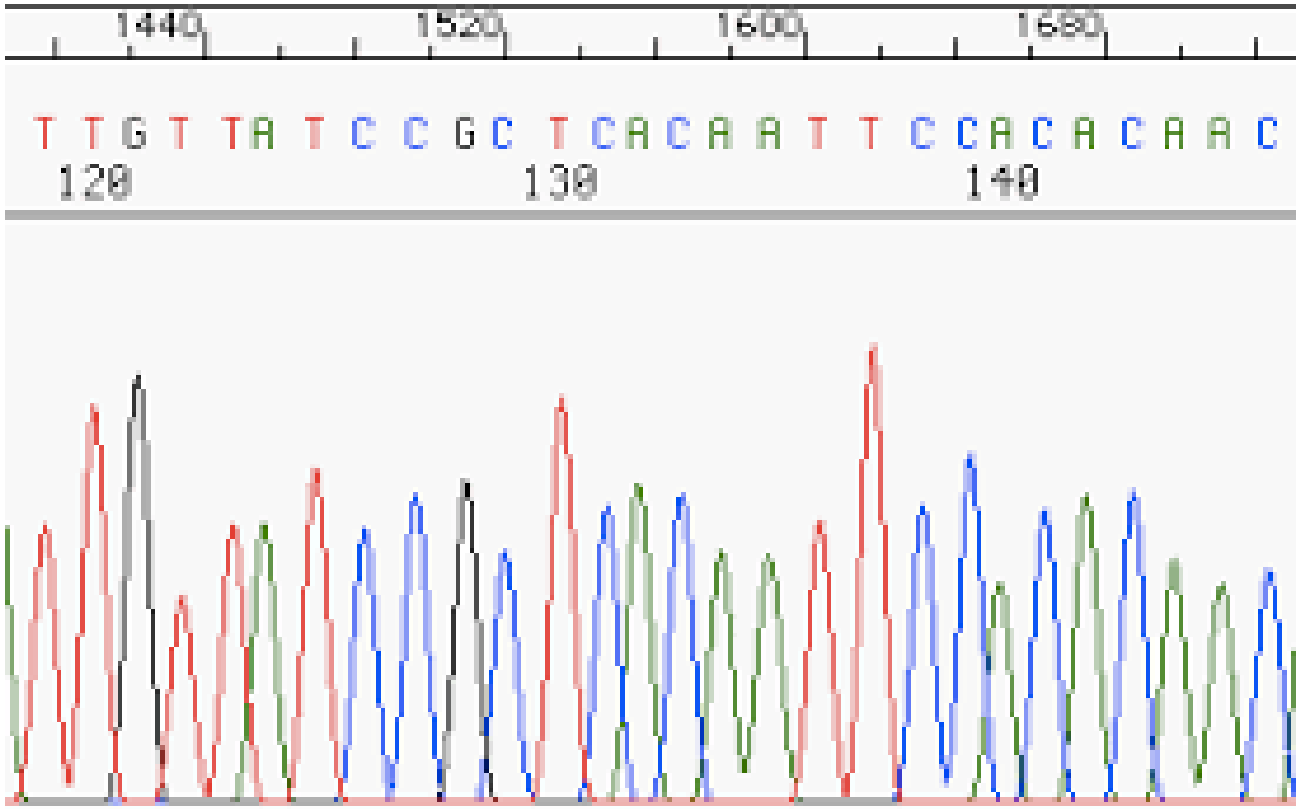
Hapsburg jaw



Ellis-Van Creveld syndrome, a sixth finger

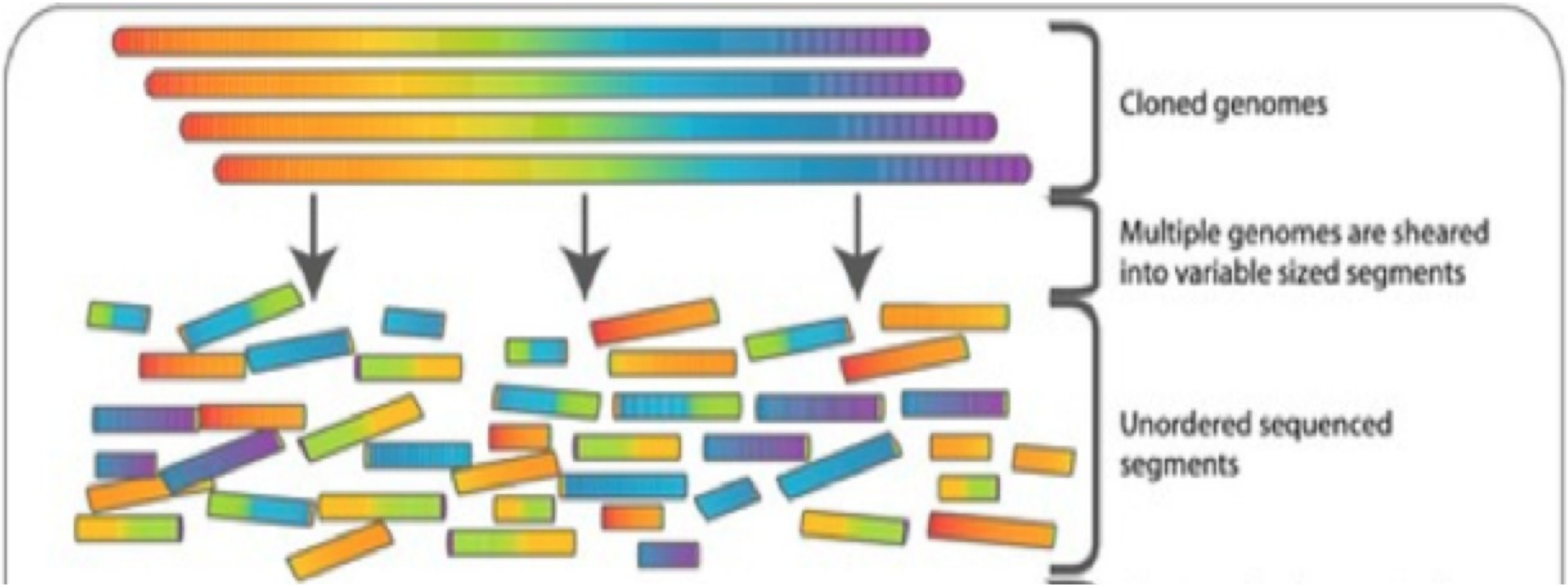
Genome Sequencing

- Bases are recorded as little peaks
- Reads = Small segments of DNA from sequencer machine
- Contigs = Segments of partially combined reads



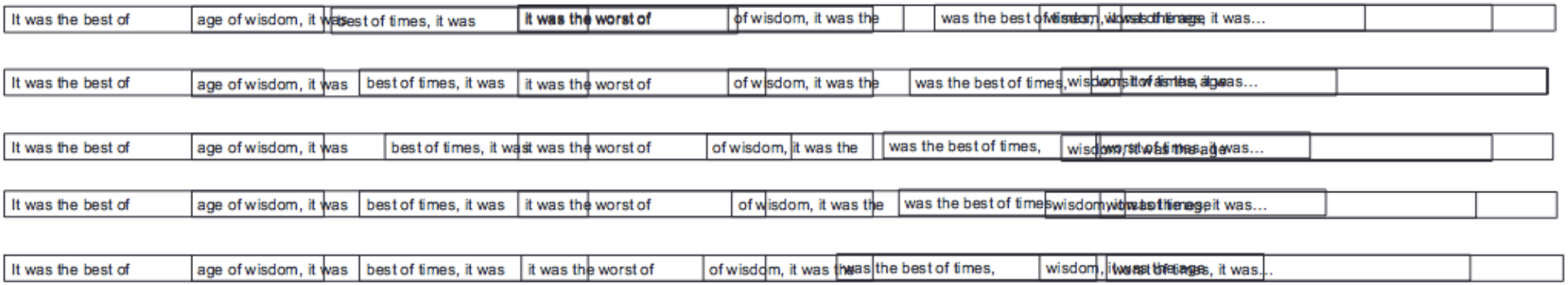
Genome Sequencing

- Combine pieces like a jigsaw puzzle



Shredded Book Reconstruction

- Dickens accidentally shreds first printing of Tale of Two Cities



times, it was the age

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Assembly Parameter:
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Tale of Two Cities
Charles Dickens

It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity, it was the season of Light, it was the season of Darkness, it was the spring of hope, it was the winter of despair, we had everything before us, we had nothing before us, we were all going direct to Heaven, we were all going direct the other way - in short, the period was so far like the present period, that some of its noisiest authorities insisted on its being received, for good or for evil, in the superlative degree of comparison only.

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Repeats pile up – actual placement of each individual fragment unknown

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Assembly Parameter:
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Repeats pile up – actual placement of each individual fragment unknown

Repeats can cause ambiguity and prevent proper assembly

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of times, it was the

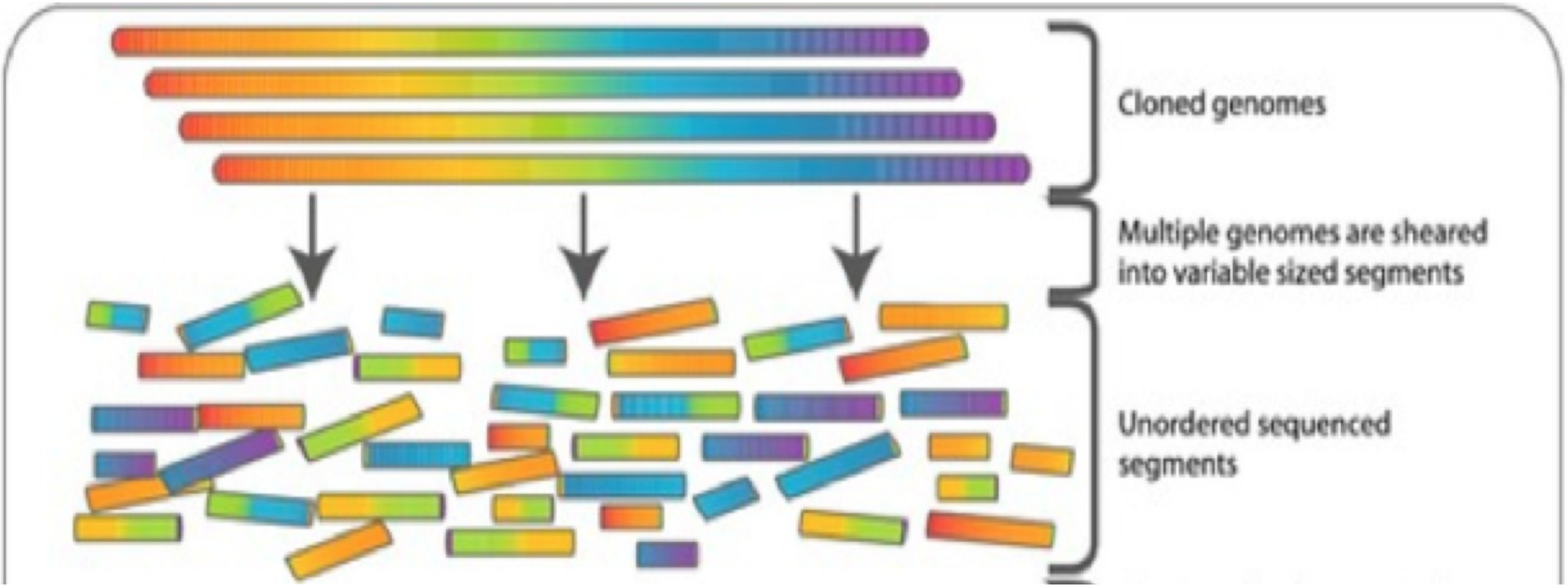
times, it was the age

times, it was the worst

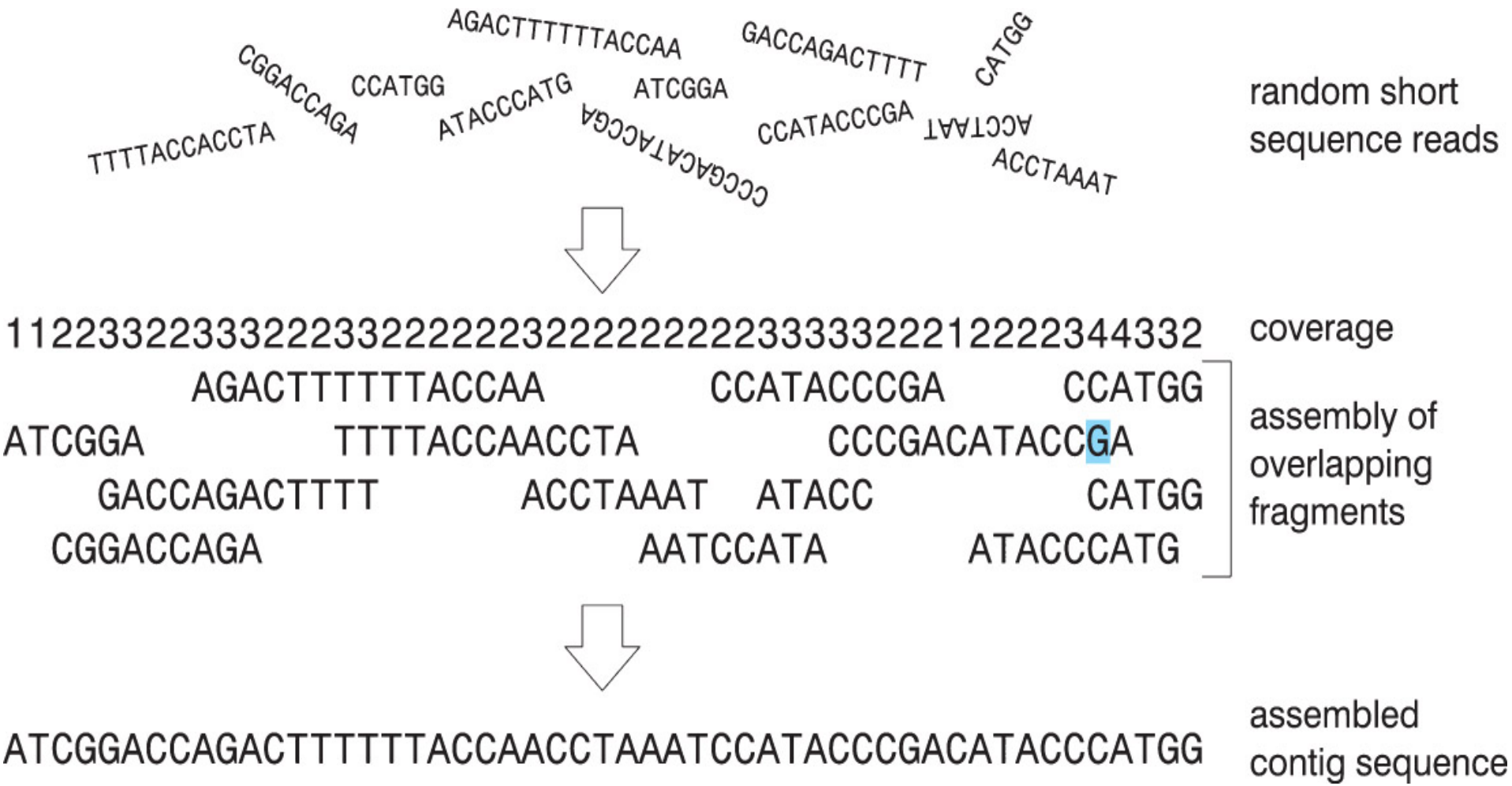
It was the best of times, it was the [age/worst]

Assembly Parameter:
100% identify across 4 words

Genome Sequencing



Coverage


AGACTTTTTTACCAA
GACCAGACTTTT
CATGG
CGGACCAGA
CCATGG
ATACCCATG
ATCGGA
TTTTACCACCTA
CCCGACATACCGA
CCATACCCGA
ACCTAAT
ACCTAAAT
random short sequence reads
112233223332223322222232222222223333322212222344332
coverage
AGACTTTTTTACCAA
CCATACCCGA
CCATGG
ATCGGA
TTTTACCAACCTA
CCCGACATACCGA
GACCAGACTTTT
ACCTAAAT
ATACC
CATGG
CGGACCAGA
AATCCATA
ATACCCATG
assembly of overlapping fragments
ATCGGACCAGACTTTTTTACCAACCTAAATCCATACCCGACATACCCATGG
assembled contig sequence

Finding the Largest Overlap

- <u>Consider two fragment assembly:</u>
  - If there is more than one overlap, choose the **longest** overlap
  - Assume the sequences are not identical
  - Assume neither sequence is a substring of the other
  - The longest **possible** overlap is length of the shorter sequence-1

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Dealing with Noisy Sequencing Data

- Sequencing errors
- Ambiguities leading to incorrect base-calling

- Modify the algorithm so that the overlap exceeds some threshold value (instead of being perfect match)
  - Check if the number of matching bases is **threshold value x n**
  - With the **threshold value** being between 0 and 1

Assembling a Contig

Table 8.3 Overlaps for a hypothetical set of sequence reads.

Fragments	Overlaps (Length)
1. TACCTTG	2 (3), 3 (1), 4 (1), 7 (1)
2. TTGAT	1 (1), 3 (3)
3. GATATGG	4 (2), 7 (1)
4. GGAG	3 (1), 7 (1)
5. CTCTA	1 (2), 6 (3)
6. CTAGT	1 (1), 2 (1)
7. GCTCT	1 (1), 2 (1), 5 (4), 6 (2)

Assembling a Contig: graph representation