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## Comparative Mapping and Genomics



By Madan Bhattacharyya, Walter Suza (ISU)



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## Introduction

Recall that every cell in a plant contains the same genetic information. The genetic information of a cell constitutes its genome. Therefore, a genome is made up of genes and their regulatory elements. The genome size varies in different species of animals and plants. For example, the human genome is 3.2 Gb while that of hexaploid wheat is 16 Gb. Certainly, a human is very different from a wheat plant. Despite having a smaller genome, a human can think and move but a wheat plant cannot. What then brings about such stark differences? To answer this question we need to compare the genomes of these two organisms for features such as gene content, organization, and function. This type of research is referred to as comparative genomics. Using bioinformatics programs, genome sequences are aligned and the alignments are examined for their evolutionary relationship. Are they homologous, or do they share a common ancestor? Comparative analysis can also be done for genomes of different strains of a species or species that are distantly related. Differences of genomes can therefore be linked to functional consequences, or phenotypes.

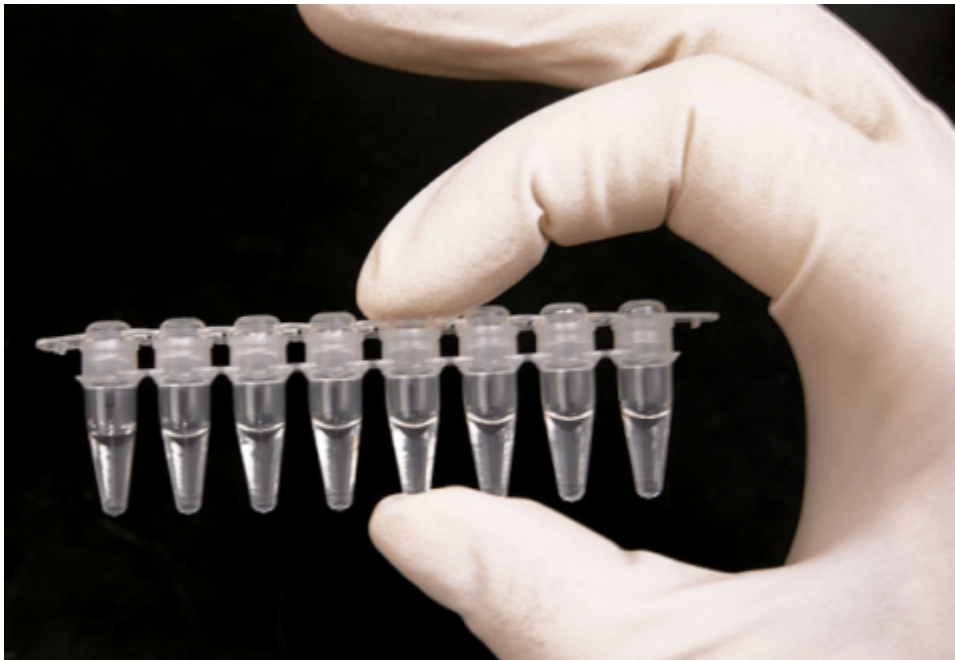
# Introduction to Structural Genomics

## Overview

To conduct comparative genomics we need to know the structure of the genomes we wish to compare. We also need tools/approaches to perform such an analysis. The following sections describe mapping concepts and the fundamentals of comparative genomics.

## Objectives

- Understand the difference between genetic and physical maps
- Familiarize with comparative genomics tools
- Understand the challenges in comparative genomics
- Application of comparative mapping



**Fig. 1 Sample tubes used in PCR analysis. Photo by Madprime. Liscensed under CC BY-SA 3.0 via Wikimedia Commons.**

## Genetic Maps

The purpose of genetic maps (also called linkage maps) is to report the length of chromosome intervals, chromosomes, and whole genomes. Genetic maps are based on the rate of recombination. Thus, genetic distances reflect the number of crossover events “observed” for the region, chromosome, or genome of interest. Figure 2 is an example of a genetic map in tomato. Compare the linkage map of molecular markers with the classical genetic map. Molecular markers are super abundant and a single cross allows mapping thousands of markers. Classical maps based on morphological markers are less dense and require integration of maps developed from many crosses. Compare the molecular map with the cytological map on the right. The markers are highly dense in the heterochromatic regions containing the centromeres. This is because of the reduced or suppressed recombination rates in the heterochromatic regions.

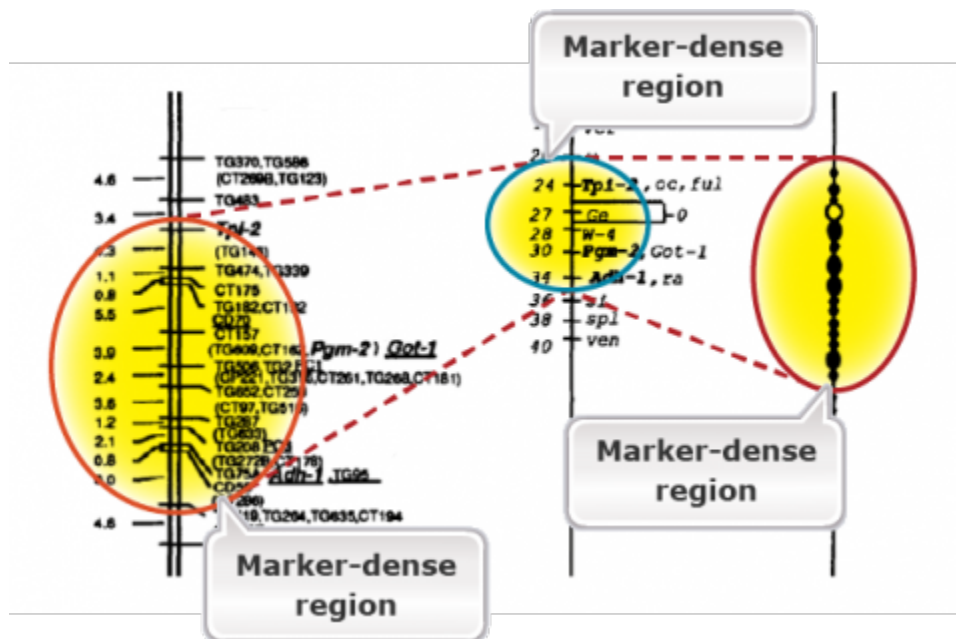


Fig. 2 Molecular linkage map of the tomato genome (left) and comparison with classical map (center) and cytological (pachytene) map (right). Adapted from Tanksley et al., 1992.

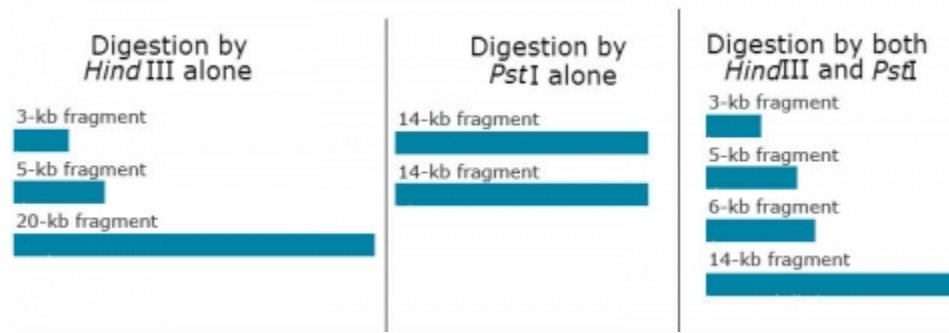


## *Physical Maps*

While genetic map is based on the rates of crossing over and is arbitrary, physical maps provide physical locations of markers. Fluorescence in situ hybridization (FISH) mapping of genetic markers on the pachytene chromosomes can allow us developing a physical map that corresponds to a genetic map (Fig. 2). Note that in Fig. 2, certain regions are expanded in the genetic map due to higher rates of recombination. Reverse is true for the heterochromatic regions including the centromeres due to reduced recombination rates. Thus, crossover events are not evenly distributed across the chromosomes. Crossover events tend to be suppressed in centromeres and repetitive DNA-rich heterochromatic regions, whereas they are enhanced generally in gene-rich, euchromatic regions. With sequencing of the entire genomes of crop species, one can now have physical maps of individual chromosomes based on nucleotide sequence. Genome browsers (e.g., [Phytozome](#) for soybean) can allow us to navigate the physical maps for gene sequences or molecular markers to the nucleotide level.

## Restriction Mapping

Restriction mapping can also allow us to generate a physical map of small DNA fragments cloned in a plasmid vector or larger fragments cloned in BAC (bacterial artificial chromosome) or YAC (yeast artificial chromosome) vectors. This requires determination of the positions of restriction sites on DNA. Consider a piece of linear DNA of 28 kb. The DNA was cut first by *Hind*III alone, then by *Pst*I alone, and, finally, by both *Hind*III and *Pst*I together. The following results were obtained:

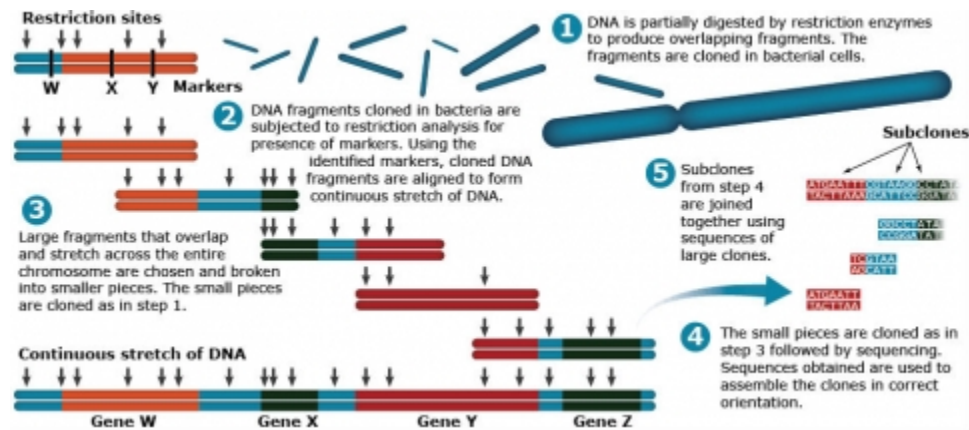


**Fig. 3 Results of DNA digestion by different enzymes.**

Using these results, draw a map of the *Hind*III and *Pst*I restriction site on this 28-kb piece of DNA, indicating the relative positions of the restriction sites and the distances between them.

## Physical Maps and Genome Sequencing

With progress in sequencing technology, an increased number of plant genomes have been sequenced. As a result, physical maps have gained importance. The assembly of the whole-genome sequence relies on both genetic and physical maps for aligning sequenced fragments. Recall in Lesson 5 that BAC and YAC clones are used to prepare genomic libraries for sequencing. The cloned DNA fragments in a YAC or BAC are aligned to form continuous stretches of DNA for subsequent sequencing processes (Fig. 4).

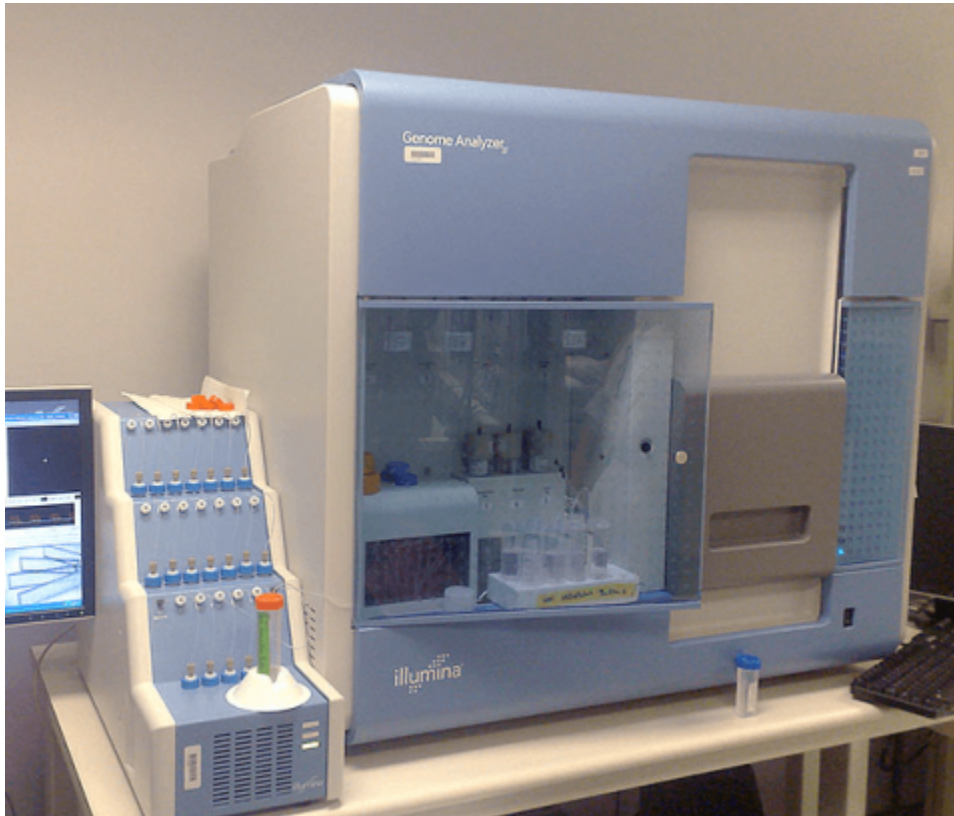


**Fig. 4** Physical maps are used to order cloned DNA fragments facilitating genome sequencing. Adapted from Pierce, 2010.

# Comparative Mapping

## *Description*

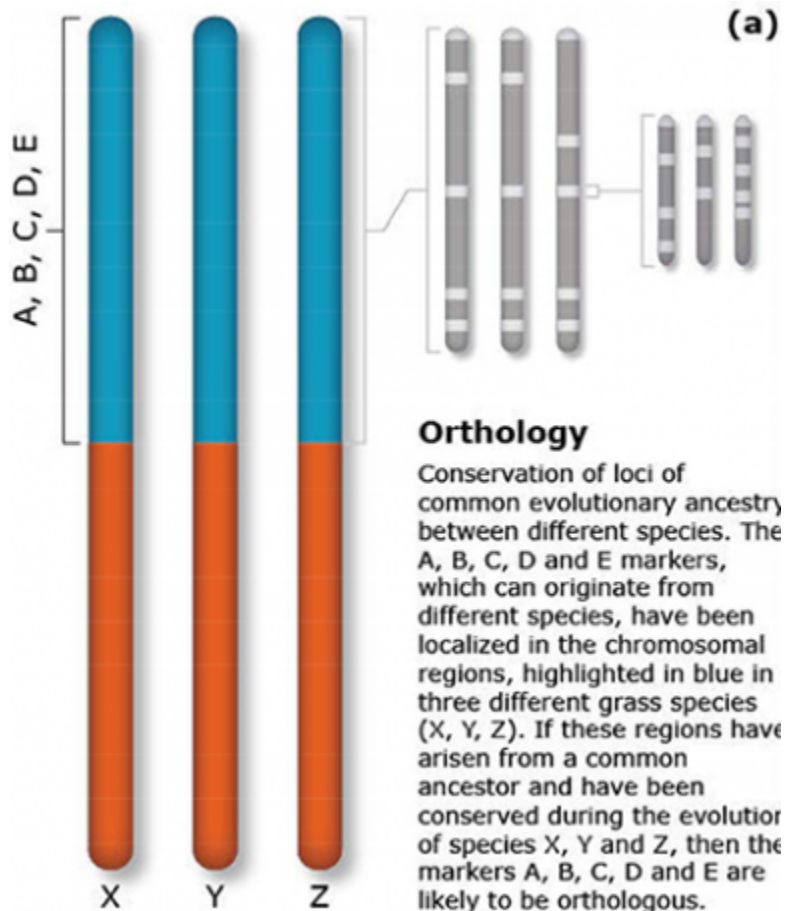
Comparative mapping is a study how the genomes relate across species and genera and even families. The concept started with comparative mapping experiments using RFLP markers between two species that led to the discovery of conserved linear orders of marker loci across related species.



**Fig. 5 The Illumina Genome Analyzer II System. Photo by Jon Callas. Licensed under CC BY 2.0 via Wikimedia Commons.**

## Colinearity and Synteny

The terms synteny and colinearity have been broadly used to describe the presence of conserved gene orders on chromosomes across species, genera or families. Colinearity describes the conservation of the gene order within a chromosomal segment between different species (Fig. 7). The term colinearity is used to explain conservation of loci at the chromosome level, and micro-colinearity at the locus level (Fig. 8). Synteny was originally used to describe the physical mapping without the linkage assumption. Now the term is used to define chromosomal segments or to gene loci in different organisms located on a chromosomal region originating from a common ancestor (Keller and Feuillet 2000). Genetic loci that arose from a common ancestor are defined as orthologous loci; whereas, paralogous loci are evolved through tandem duplication within a species and located side by side in a chromosomal segment. The examples of colinearity and micro-colinearity are shown in Figures 7 and 8, respectively.



**Fig. 6 Different levels of conservation between the grass genomes: Orthology. Adapted from Trends in Plant Science.**

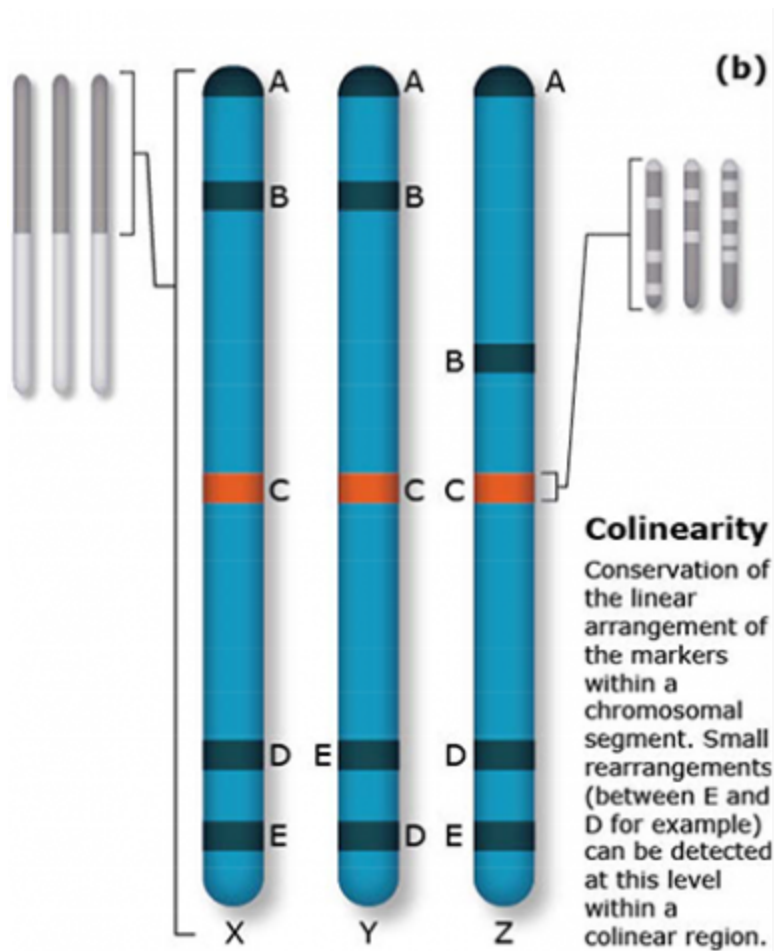
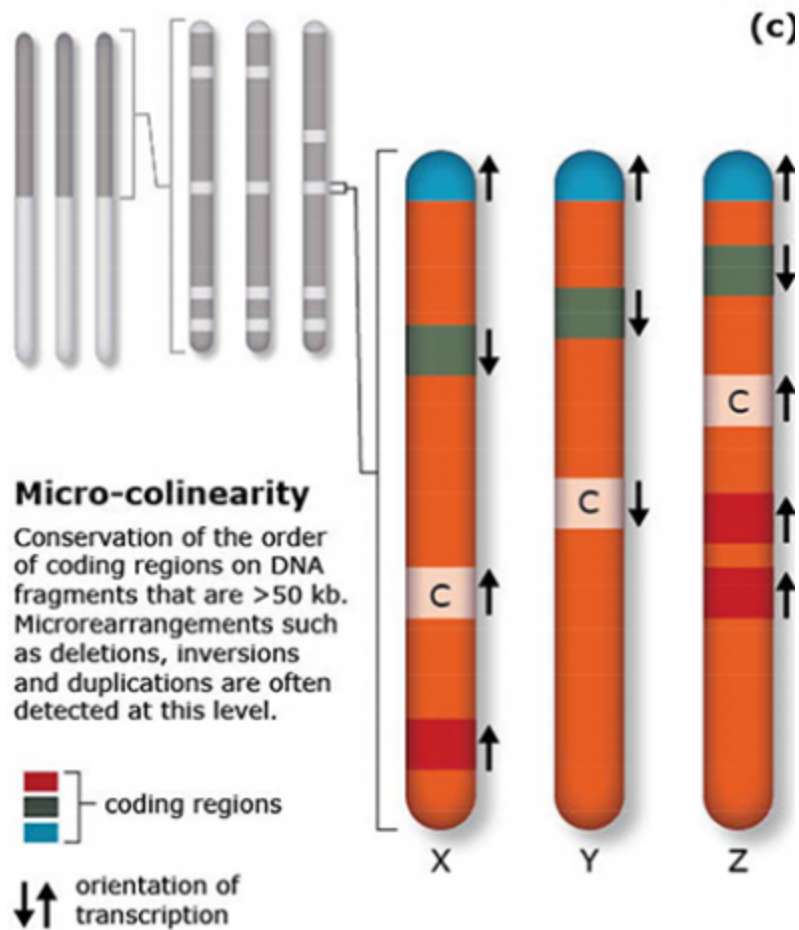


Fig. 7 Different levels of conservation between the grass genomes: Colinearity. Adapted from Trends in Plant Science.

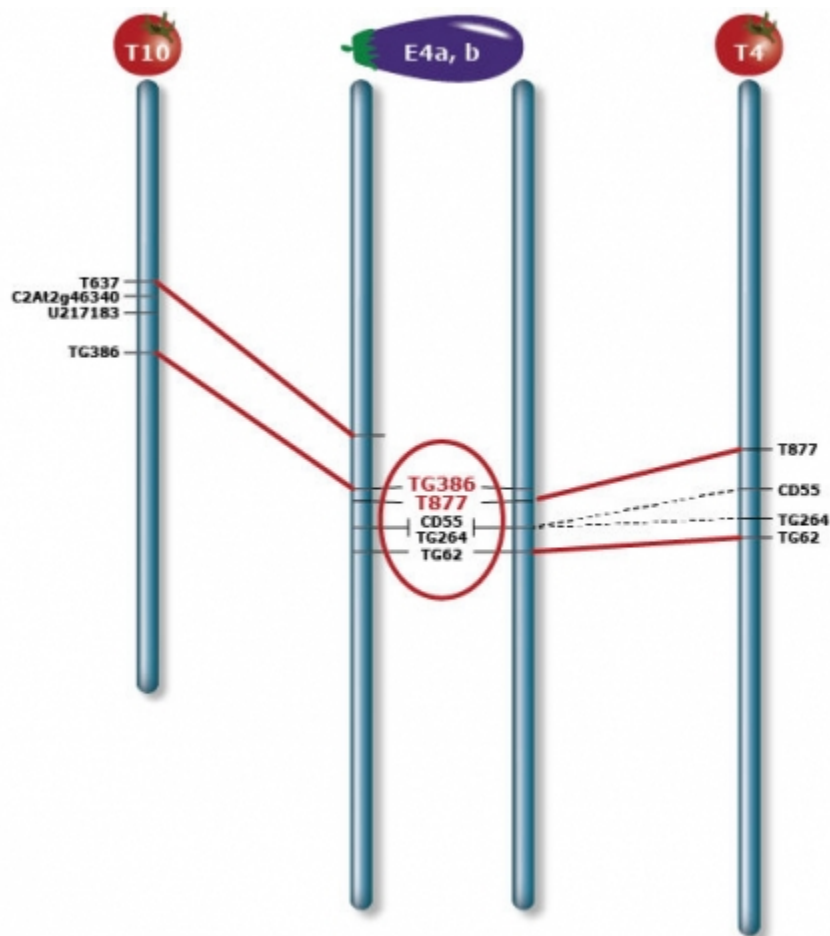


**Fig. 8 Different levels of conservation between the grass genomes: Micro-colinearity.** Adapted from Trends in Plant Science.



## Orthology Example

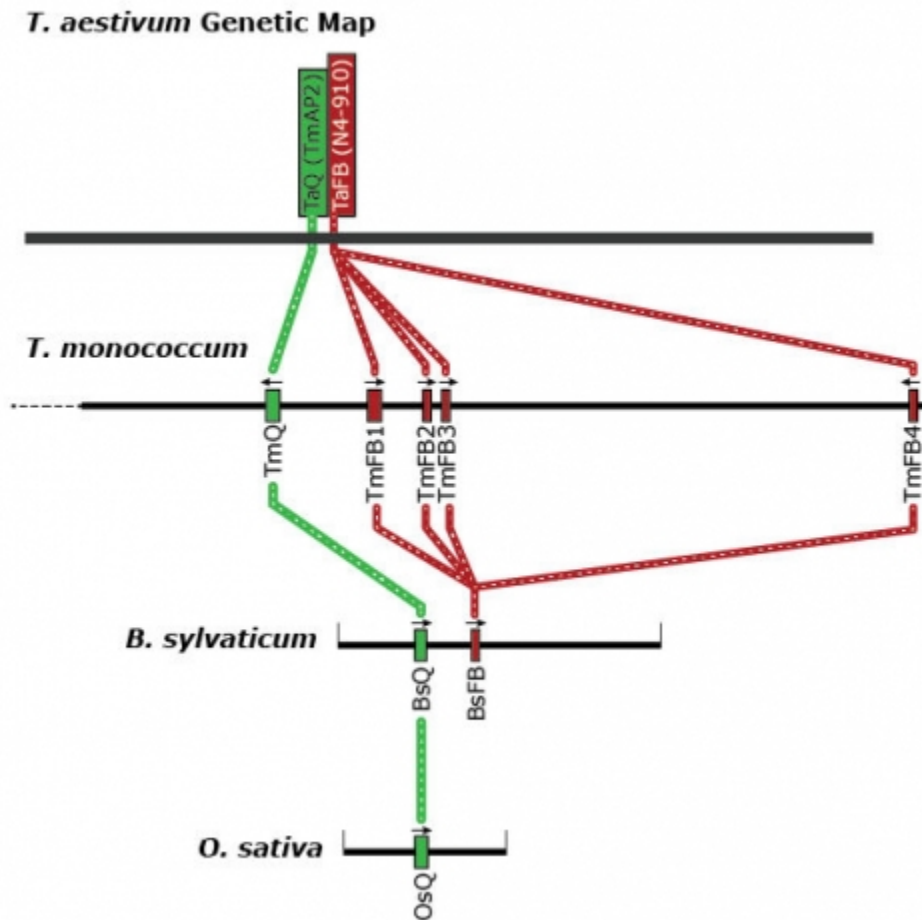
The eggplant chromosome E4 combines two segments (E4a and E4b) orthologous to tomato T4 and T10 respectively, indicating a translocation between the two genomes. The breakpoint is located between markers TG386 and T677 (highlighted in red), and the region is indicated by a black bar beside E4. Orthologous marker pairs are connected by lines. A dash line indicates a marker of low mapping confidence on either or both maps that is not used for deduction of inversions. Vertical arrows beside E4 depict inversions in E4 with respect to T10.



**Fig. 9 Chromosomal rearrangement between genomes of eggplant and tomato.** Adapted from Wu and Tanksley, 2010.

## Micro-Colinearity Example

The genetic map of bread wheat (*Triticum aestivum*) is used to analyze micro-colinearity of the Q locus of *T. monococcum*, *Brachypodium sylvaticum*, and rice (*Oryza sativa*). Genes are shown as colored boxes along the physical maps of each species.



**Fig. 10** The genetic map of bread wheat (*Triticum aestivum*) is used to analyze micro-colinearity of the Q locus of *T. monococcum*, *Brachypodium sylvaticum*, and rice (*Oryza sativa*). Selected genes are shown as colored boxes along the physical maps of each species, and transcriptional orientations are indicated by arrows above the boxes.

## *Orthology and Mapping*

Comparative mapping is the alignment of chromosomes of related species based on genetic mapping of common DNA markers. Thus, comparative mapping involves the development of linkage maps (**Fig. 1**). The construction of comparative maps depends on orthology predictions to identify gene pairs of two species. Orthologous loci are loci in different species originating from the same ancestral locus. In contrast, paralagous loci are loci in different (or the same) species that arose due to a duplication of an ancestral locus.

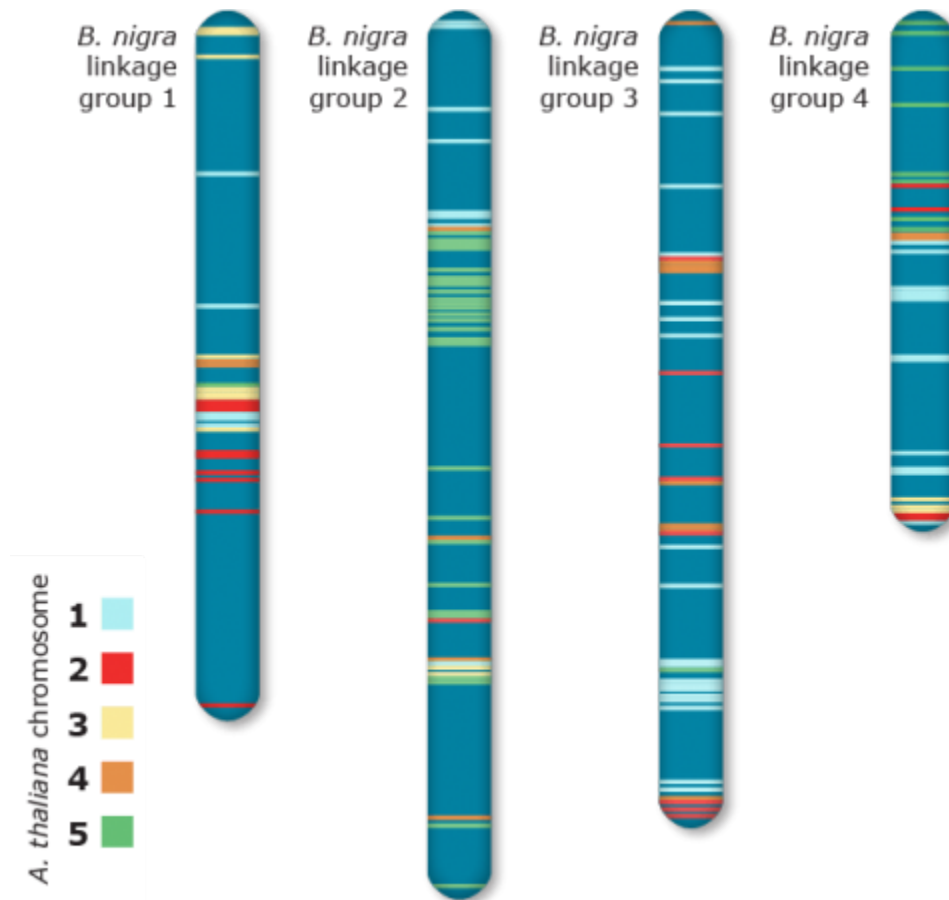
Once the gene pairs have been established, blocks of conserved syteny are established using the positions of each gene in their respective map. The comparative studies in Solanaceae species revealed a modest and consistent rate of chromosomal changes across the family (0.03 ~ 0.12 rearrangements per chromosome per million years). Closely related species showed more conservation of gene orders than the distantly related species. For example, a high conservation of marker orders was observed between tomato and eggplant or tomato and potato than between tomato and pepper. Also, hot spots of chromosomal breakages were identified to suggest that breakpoints are not randomly distributed across the genome. In general, a higher frequency of inversions than translocations was observed among the Solaneaceous species.

## *Grass Genome Map*

Early research to evaluate synteny in grass species suggested the grouping of grasses of the Poaceae families as a single genetic system (Bennetzen and Freeling, 1993). This early synteny work revealed that a large degree of colinearity exists among diverse grasses. For instance, a high conservation across grass species was observed in regions ranging from 5-10 cM. Also, most genes are homologous across species, i.e. all species have essentially the same genes. Additional fine structure mapping revealed insertions of repeated sequences among grass genomes. Overall, these efforts led to the development of the circular grass genome map.

## Linear Comparative Map

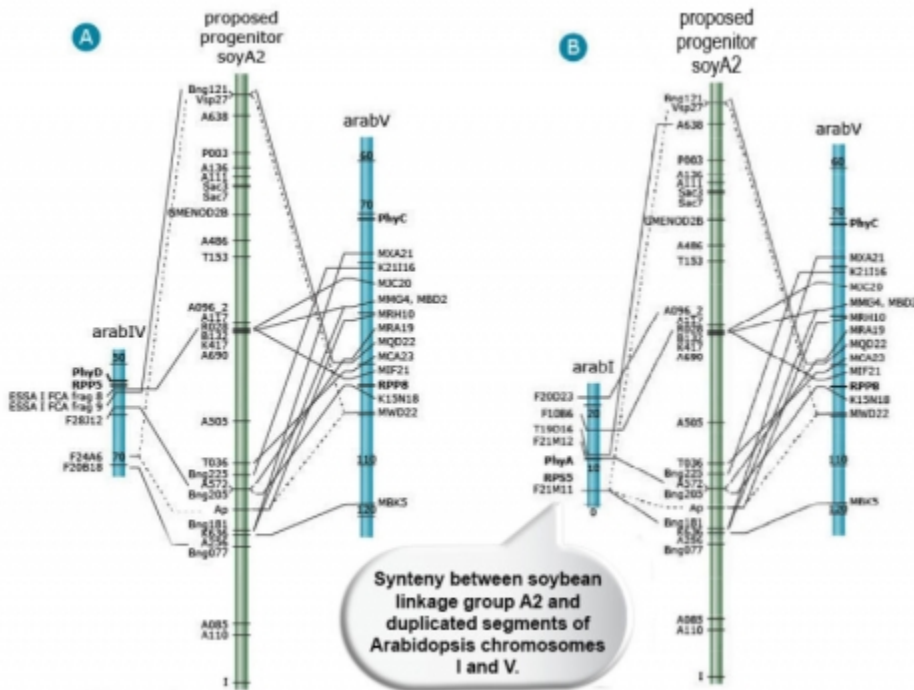
The average conserved segments between *Arabidopsis* and *B. nigra* was estimated to be ~8 cMs (Fig. 11). This estimate corresponds to ~90 rearrangements since divergence of the two species; much higher than other species.



**Fig. 11** A linear comparative map of *Arabidopsis thaliana* and *Brassica nigra*. Four out of eight linkage groups (G1-G4) of *B. nigra* are represented by vertical lines. The chromosomal location of *A. thaliana* loci detected by the *A. thaliana* markers are shown with different colors. Adapted from Lagercrantz, 1998.

## Soybean and Arabidopsis Linkage

The majority of the comparative mapping studies were based on conservation of nucleotide sequences among closely related species. In 2000, synteny between soybean and *Arabidopsis* chromosomes was observed when linear orders of predicted protein sequences of genes were compared between the two species (Figure 12). This study also showed that *Arabidopsis* contains large scale duplicated genomic regions (Grant et al. 2000).



**Fig. 12 Only those loci that had significant homology to Arabidopsis sequences on arabI or arabV are connected by lines, although tic marks for every soybean sequence analyzed are shown on the proposed progenitor soyA2 map. Thin lines indicate soybean sequences that had homologs on only one Arabidopsis chromosome. Broken lines are used to indicate uncertainty in syntenic relationships because of duplicated loci in soybean. Known genes in Arabidopsis are shown in bold type. Tic marks and numbers indicate 10-cM intervals on the Arabidopsis chromosomes. Adapted from Grant et al., 2000.**

## Web-Based Mapping Tools

Web-based applications are available for mapping purposes. For example, the [Comparative Map Viewer](#) (CMap) available from GRAMENE (Fig. 13) allows comparisons of different maps.

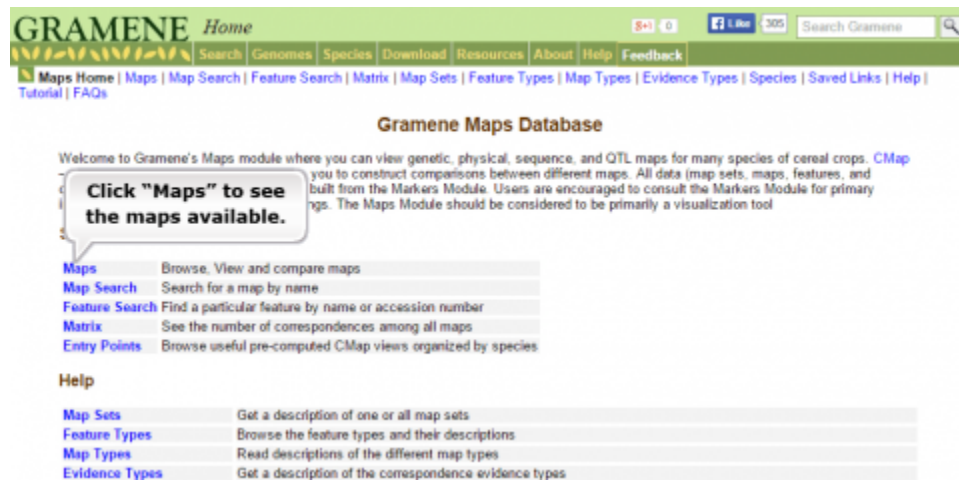


Fig. 13 Gramene's Maps module allows viewing of genetic, physical, and comparative maps for cereal crops.

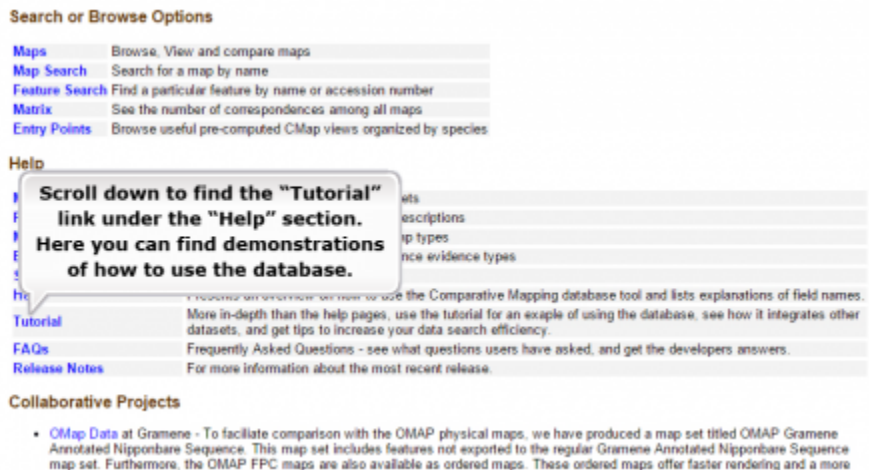


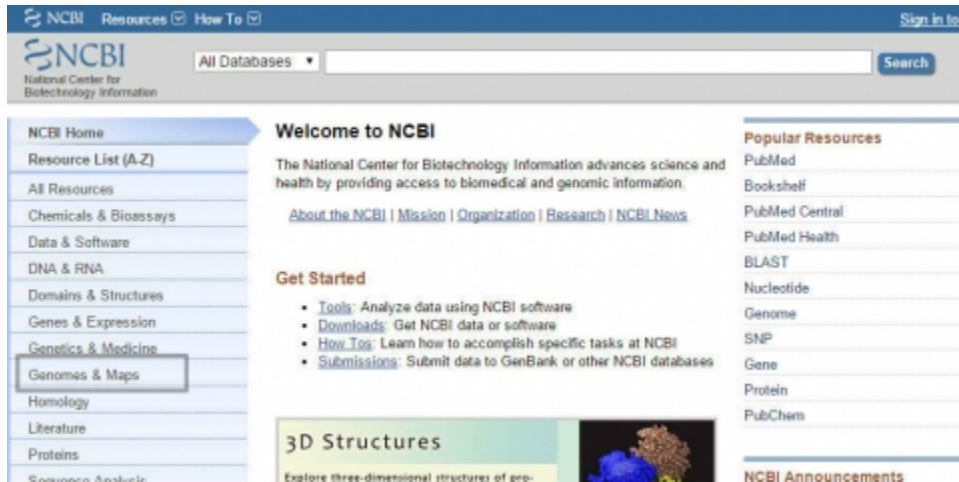
Fig. 14 Gramene's Maps module allows viewing of genetic, physical, and comparative maps for cereal crops.

[Try This: Using the NCBI MapViewer](#)



## Try This: NCBI MapViewer

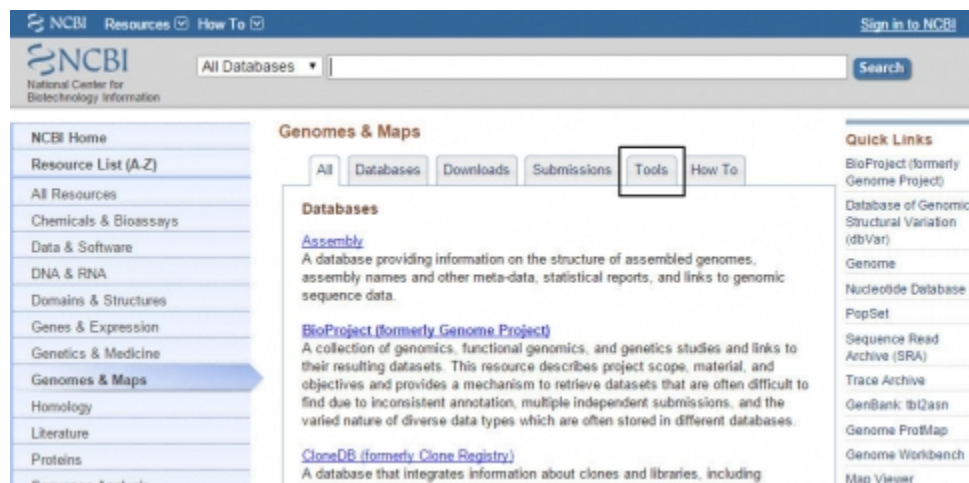
In "Introduction to Bioinformatics" module, you learned about bioinformatic webtools. Go to [NCBI](https://www.ncbi.nlm.nih.gov) and access MapViewer within Genomes & Maps.



The screenshot shows the NCBI homepage. At the top, there is a navigation bar with 'NCBI', 'Resources', and 'How To' links, along with a 'Sign in to f' link. Below this is a search bar with a dropdown menu set to 'All Databases' and a 'Search' button. The main content area is divided into three columns. The left column is a sidebar with a list of categories: 'NCBI Home', 'Resource List (A-Z)', 'All Resources', 'Chemicals & Bioassays', 'Data & Software', 'DNA & RNA', 'Domains & Structures', 'Genes & Expression', 'Genetics & Medicine', 'Genomes & Maps' (highlighted with a blue box), 'Homology', 'Literature', 'Proteins', and 'Species & Strains'. The middle column is titled 'Welcome to NCBI' and contains a paragraph about the center's mission, a list of links ('About the NCBI', 'Mission', 'Organization', 'Research', 'NCBI News'), and a 'Get Started' section with links for 'Tools', 'Downloads', 'How To', and 'Submissions'. The right column is titled 'Popular Resources' and lists various tools and databases: 'PubMed', 'Bookshelf', 'PubMed Central', 'PubMed Health', 'BLAST', 'Nucleotide', 'Genome', 'SNP', 'Gene', 'Protein', and 'PubChem'. At the bottom of the right column is a section for 'NCBI Announcements'.

## Try This: NCBI MapViewer

In "Introduction to Bioinformatics" module, you learned about bioinformatic webtools. Go to [NCBI](https://www.ncbi.nlm.nih.gov) and access MapViewer within Genomes & Maps.



The screenshot shows the NCBI website interface. At the top, there is a navigation bar with 'NCBI', 'Resources', and 'How To' links, along with a 'Sign in to NCBI' button. Below this is a search bar with a dropdown menu set to 'All Databases' and a 'Search' button. On the left side, there is a vertical menu with various categories, including 'Genomes & Maps', which is currently selected and highlighted in blue. The main content area is titled 'Genomes & Maps' and features a sub-navigation bar with tabs for 'All', 'Databases', 'Downloads', 'Submissions', 'Tools', and 'How To'. The 'Tools' tab is highlighted with a black border. Below the tabs, the 'Databases' section is visible, listing several databases: 'Assembly', 'BioProject (formerly Genome Project)', and 'CloneDB (formerly Clone Registry)'. On the right side of the page, there is a 'Quick Links' section with a list of links to various NCBI resources, including 'BioProject (formerly Genome Project)', 'Database of Genomic Structural Variation (dbVar)', 'Genome', 'Nucleotide Database', 'PopSet', 'Sequence Read Archive (SRA)', 'Trace Archive', 'GenBank: t2asn', 'Genome ProfMap', 'Genome Workbench', and 'Map Viewer'.

## Try This: NCBI MapViewer

In "Introduction to Bioinformatics" module, you learned about bioinformatic webtools. Go to [NCBI](#) and access MapViewer within Genomes & Maps.

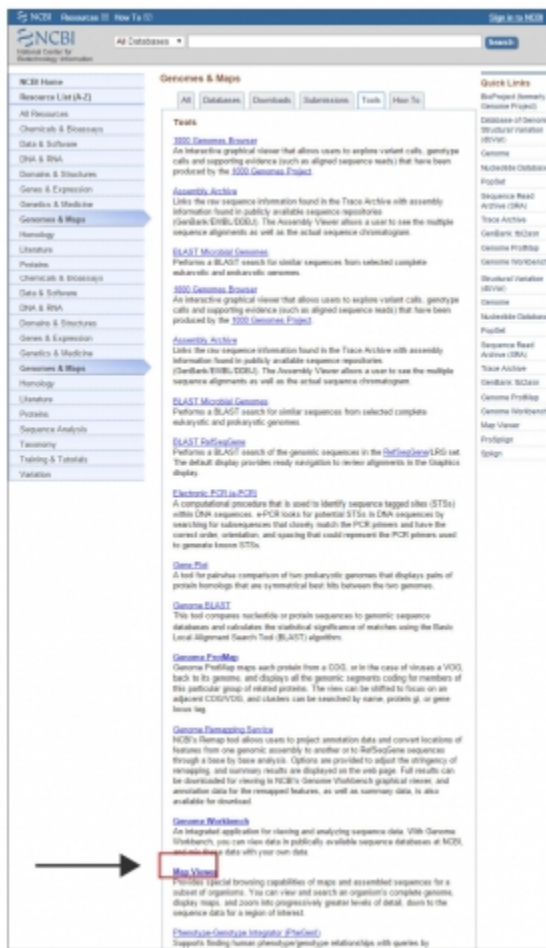


Fig. 15 Screenshot of NCBI Map Viewer

## Try This: NCBI MapViewer

Search for plants, and select *Phaseolus vulgaris* (kidney bean).

NCBI Home GenBank BLAST

Map Viewer Home Help

The Map Viewer provides a wide variety of genome mapping and sequencing data. [More...](#)

Search:    
 for:

Tools Legend

- Search or Browse the Genome
- BLAST
- Clone Finder
- Go to region on a chromosome
- Genome Resources page

News

22 new annotation releases added to MapViewer Jan 6, 2014  
The following 22 Annotation Releases have been added to MapV... [more](#)

Five plant annotation releases Dec 18, 2013

Scientific name	Common name	Build	Tools
<b>Vertebrates</b> (147)			
<b>Mammals</b> (74)			
<b>Primates</b> (15)			
<i>Callithrix jacchus</i>	white-tufted-ear marmoset	<a href="#">Annotation Release 102</a>	
<i>Chlorocebus sabaeus</i>	green monkey	<a href="#">Annotation Release 100</a>	
<i>Gorilla gorilla</i>	western gorilla	<a href="#">Annotation Release 100</a>	
<i>Homo sapiens</i>	human	<a href="#">Annotation Release 106</a>	
		<a href="#">Annotation Release 105</a>	
<i>Macaca fascicularis</i>	crab-eating macaque	<a href="#">Annotation Release 100</a>	
<i>Macaca mulatta</i>	rhesus macaque	<a href="#">Build 1.2</a>	
<i>Nomascus leucogenys</i>	northern white-cheeked gibbon	<a href="#">Annotation Release 101</a>	
		<a href="#">Build 1.1</a>	
<i>Otolemur garnettii</i>	small-eared galago	<a href="#">Annotation Release 100</a>	
<i>Pan paniscus</i>	pygmy chimpanzee	<a href="#">Annotation Release 101</a>	
<i>Pan troglodytes</i>	chimpanzee	<a href="#">Annotation Release 103</a>	
		<a href="#">Annotation Release 102</a>	
<i>Papio anubis</i>	olive baboon	<a href="#">Annotation Release 101</a>	

## Try This: NCBI MapViewer

The screenshot shows the NCBI MapViewer interface. At the top, there's a navigation bar with links to PubMed, Nucleotide, Protein, Genome, and Genes. Below this is a search bar with the text "Search for" and a dropdown menu showing "on chromosome(s)". The main content area displays the title "Phaseolus vulgaris (kidney bean) genome Build 0.1 statistics" in purple. Below the title is a bar chart showing the estimated length of each chromosome. The chromosomes are numbered 1 through 11, with MT and Pltd also shown. The bars represent the estimated length of each chromosome, with chromosome 1 being the longest and chromosome 11 being the shortest. The MT and Pltd bars are significantly shorter than the others.

NCBI

PubMed Nucleotide Protein Genome Genes

Search for  on chromosome(s)

Map Viewer

Map Viewer Home

Map Viewer Help

Plant search

NCBI Resources

Genome Project

Organism Data in GenBank

EST

Sequencing Projects

*Phaseolus vulgaris (kidney bean) genome Build 0.1 statistics*

1 2 3 4 5 6 7

8 9 10 11 MT Pltd

How many chromosomes does kidney bean have?

Select chromosome 1, and answer the following:

- How many maps are available for chromosome 1?
- Are the maps physical or genetic? What is the estimated length for each map?
- List the types of markers used to develop each map?
- Try the zoom in function in the left corner, what do you see?

# Comparative Genomics

## *Description*

With the advent of nextgen sequencing there has been a continuous supply of genome sequence data in the literature. Now the concept of comparing genome maps looking for linear order of genes or synteny has been changed to comparative genomics. It is now feasible to compare related or distant species or genera at the genome level with the aid of available genome sequences. Comparative genomics will have impact in advancing our knowledge not only in evolution of crop species, but also in answering biological questions. For example, traditional studies on domestication traits were focused on dozens of loci involved in a variety of functions. Many of the traits were not amenable to study using conventional mapping approaches. Through comparative genomics it is now known that about 24% of loci in the maize genome were involved in either domestication or subsequent improvement. Through comparative genomics studies it is now known that in both maize and sunflowers there some loci related to amino acid biosynthesis are enriched. Selection of genes for amino acid biosynthesis during domestication may suggest that protein metabolism has an important role in heterosis. In barley, allelic variation at a flowering time locus in European cultivars appears to have arisen by introgression from barley that was independently domesticated in Central Asia.

## *Gene Prediction*

The availability of genome sequence information makes it possible to apply comparative genomics for identification of genes. Gene prediction by comparative analysis involves identification of local similarities by sequence alignment programs in pairs of closely or distantly related genomes. For example, the mouse genome helped increase the accuracy of predicting human genes (Parra et al. 2003).

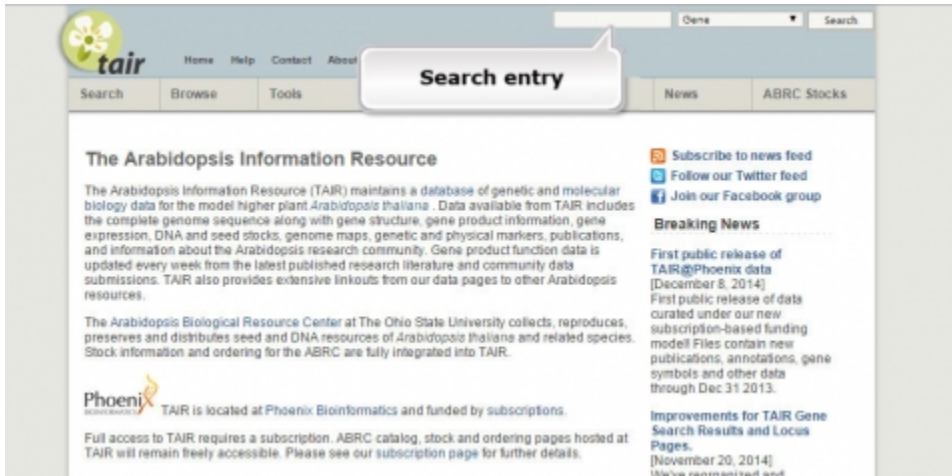


**Fig. 16 Analysis of the mouse genome helped increase the accuracy of predicting human genes. Photo licensed under CC BY-SA 3.0 via Wikimedia Commons.**



## Try This: Arabidopsis Analysis

Go to the [Arabidopsis Information Resource](#) and search for a gene called JAR1.



The screenshot shows the homepage of the Arabidopsis Information Resource (TAIR). At the top, there is a navigation bar with links for Home, Help, Contact, and About. A search bar is located in the top right corner, with a dropdown menu set to "Gene" and a "Search" button. Below the navigation bar, there is a "Search entry" button. The main content area is titled "The Arabidopsis Information Resource" and contains a detailed description of the resource. To the right of the main content, there are links to subscribe to the news feed, follow the Twitter feed, and join the Facebook group. Below these links, there is a "Breaking News" section with two entries: "First public release of TAIR@Phoenix data [December 8, 2014]" and "First public release of data curated under our new subscription-based funding model! Files contain new publications, annotations, gene symbols and other data through Dec 31 2013." At the bottom of the page, there is a section for "Improvements for TAIR Gene Search Results and Locus Pages" dated November 20, 2014, mentioning reorganization.

**tair** Home Help Contact About

Search Browse Tools **Search entry** News ABRC Stocks

**The Arabidopsis Information Resource**

The Arabidopsis Information Resource (TAIR) maintains a database of genetic and molecular biology data for the model higher plant *Arabidopsis thaliana*. Data available from TAIR includes the complete genome sequence along with gene structure, gene product information, gene expression, DNA and seed stocks, genome maps, genetic and physical markers, publications, and information about the Arabidopsis research community. Gene product function data is updated every week from the latest published research literature and community data submissions. TAIR also provides extensive linkouts from our data pages to other Arabidopsis resources.

The Arabidopsis Biological Resource Center at The Ohio State University collects, reproduces, preserves and distributes seed and DNA resources of *Arabidopsis thaliana* and related species. Stock information and ordering for the ABRC are fully integrated into TAIR.

**Phoenix** TAIR is located at Phoenix Bioinformatics and funded by subscriptions.

Full access to TAIR requires a subscription. ABRC catalog, stock and ordering pages hosted at TAIR will remain freely accessible. Please see our subscription page for further details.

Subscribe to news feed  
Follow our Twitter feed  
Join our Facebook group

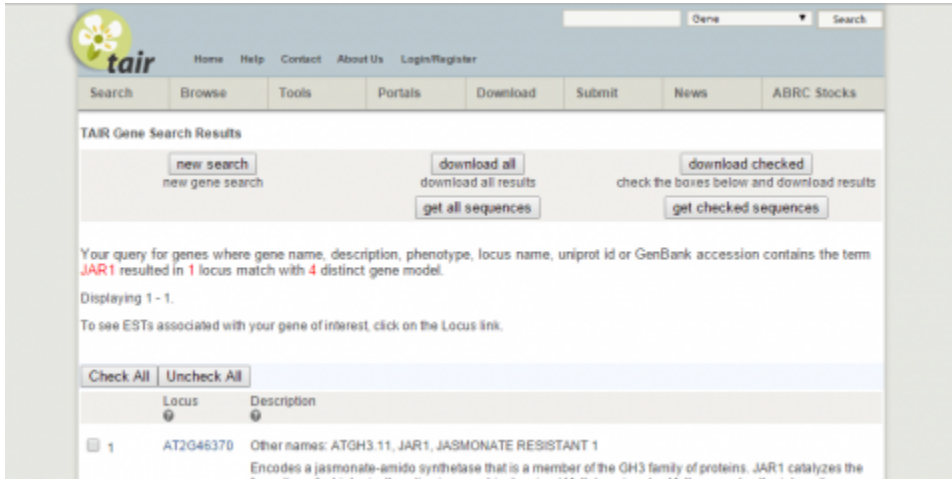
**Breaking News**

First public release of TAIR@Phoenix data [December 8, 2014]  
First public release of data curated under our new subscription-based funding model! Files contain new publications, annotations, gene symbols and other data through Dec 31 2013.

Improvements for TAIR Gene Search Results and Locus Pages.  
[November 20, 2014]  
We've reorganized and

## Try This: Arabidopsis Analysis

What is the function of JAR1 (AT2G46370)?



The screenshot shows the TAIR (The Arabidopsis Information Resource) Gene Search Results page. The page has a header with the TAIR logo and navigation links: Home, Help, Contact, About Us, Login/Register. Below the header is a search bar with the text "Gene" and a "Search" button. The main content area is titled "TAIR Gene Search Results" and contains several buttons: "new search", "download all", "download checked", "get all sequences", and "get checked sequences". The text below the buttons states: "Your query for genes where gene name, description, phenotype, locus name, uniprot id or GenBank accession contains the term **JAR1** resulted in **1** locus match with **4** distinct gene model. Displaying 1 - 1. To see ESTs associated with your gene of interest, click on the Locus link." Below this text is a table with two columns: "Locus" and "Description". The table contains one row with the following data:

Locus	Description
1 AT2G46370	Other names: ATGH3.11, JAR1, JASMONATE RESISTANT 1 Encodes a jasmonate-amido synthetase that is a member of the GH3 family of proteins. JAR1 catalyzes the

## Try This: Arabidopsis Analysis

Go to [NCBI](https://www.ncbi.nlm.nih.gov/) and search for nucleotide sequence for JAR1 (NM\_001202828.1).

The screenshot shows the NCBI Nucleotide search interface. The search term "JAR1" is entered in the search bar. The results page displays 57 nucleotide sequences. A summary box indicates that JAR1 is the jasmonic acid-amido synthetase in the Gene database, with 4 transcripts and 4 proteins. The first result is "Arabidopsis thaliana jasmonic acid-amido synthetase JAR1 mRNA" (NM\_001202828.1), which is 2,329 bp linear mRNA. The page also shows a list of top organisms, including Micrococcus mittermeieri (12), Brassica rapa (11), Camelina sativa (9), Arabidopsis thaliana (7), Tarenaya hassleriana (2), and All other taxa (15). The "Find related data" section is visible at the bottom right.

NCBI Resources How To Sign in to NCBI

Nucleotide Nucleotide JAR1 Search Save search Advanced Help

Show additional filters Display Settings: Summary, 20 per page, Sorted by Default order Send to: Filters: Manage Filters

Species  
Animals (16)  
Plants (30)  
Bacteria (1)  
More ...

Molecule types  
genomic DNA/RNA (42)  
mRNA (14)  
More ...

Source databases  
GenBank (22)  
RefSeq (33)  
More ...

Genetic

Found 57 nucleotide sequences. Nucleotide (56) EST (1)

See [JAR1](#) jasmonic acid-amido synthetase [JAR1](#) in the Gene database  
[jar1](#) reference sequences [Transcript \(4\)](#) [Protein \(4\)](#)

Results: 1 to 20 of 56 << First < Prev Page 1 of 3 Next > Last >>

1. [Arabidopsis thaliana jasmonic acid-amido synthetase JAR1 mRNA](#)  
[complete cds](#)  
2,329 bp linear mRNA  
Accession: NM\_001202828.1 GI: 334184934  
[GenBank](#) [FASTA](#) [Graphics](#)

2. [Arabidopsis thaliana jasmonic acid-amido synthetase JAR1 mRNA](#)

Results by taxon

Top Organisms [\[Tree\]](#)  
[Micrococcus mittermeieri](#) (12)  
[Brassica rapa](#) (11)  
[Camelina sativa](#) (9)  
[Arabidopsis thaliana](#) (7)  
[Tarenaya hassleriana](#) (2)  
[All other taxa](#) (15)  
More...

Find related data  
Database: [Select](#)  
[Find items](#)

### *Try This: Arabidopsis Analysis*

Within NCBI, perform a BLAST search for sequences similar to JAR1 (remember to exclude *Arabidopsis thaliana* in your query). After obtaining sequences producing significant alignments evaluate the four top sequences (maximum identity of 85-99 %). Use your results to answer the following:

- Give the name of the organism from which the sequence was obtained
- Provide the title of the research and the name of the Journal that published the research
- List GenBank id or NCBI reference sequence number of each hit

## *Detecting Copy Number Variations*

Traditional view of comparative genomics was the analysis of synteny (gene order) and sequence comparisons among related species. With the emergence of powerful computational approaches, the examination of the genomic distribution of large insertions and deletions (indels) and copy number variants (CNVs) are becoming the norm.

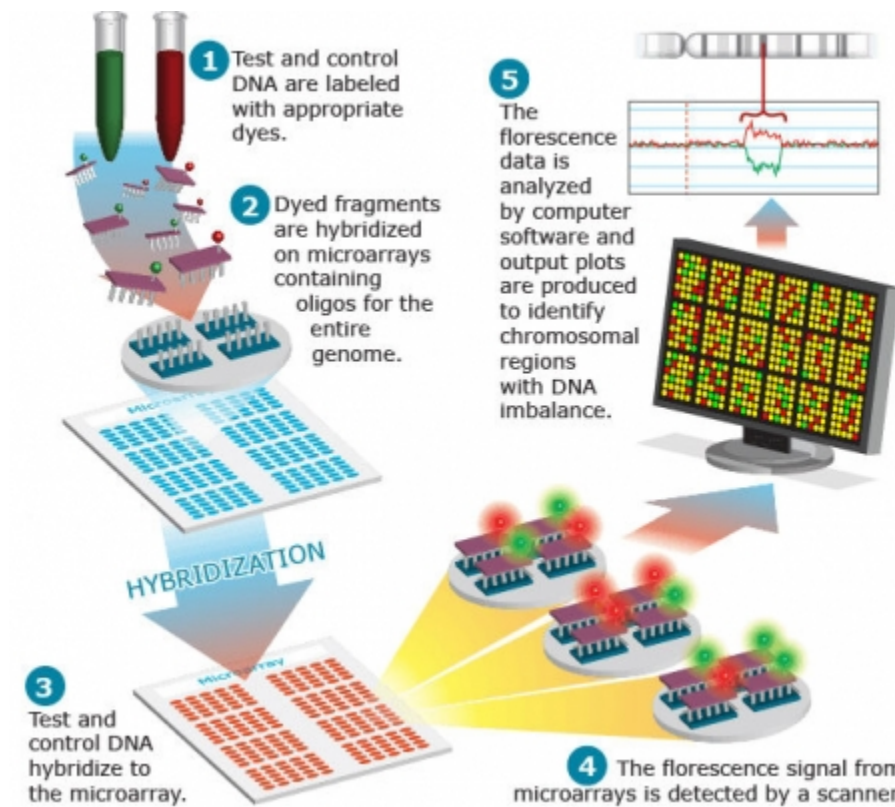
Copy number variations may result from deletions, causing some individuals to contain only a single copy of a DNA sequence, or may be due to duplications, having certain individuals with more than two copies.



**Fig. 17 A technician places a strip of eight PCR tubes into a thermal cycler at the University of Tartu in Estonia. Photo by Karl Mumm. Licensed under CC BY-SA 3.0 via Wikimedia Commons.**

## Detecting DNA Copy Number Variations

Comparative genomic hybridization (CGH) is a method for genome-wide screening for DNA copy number variations. CGH uses two genomes, a test and a control, which are labeled differentially with fluorescence probes and allowed to competitively hybridize to metaphase chromosomes. The fluorescence signal intensity from test samples compared to controls is plotted across each chromosome, allowing detection of copy number variation. Array-based CGH does not use metaphase chromosomes. Instead, synthetic oligonucleotide probes, or fragments from genomic clones such as BAC or YAC clones are arrayed onto glass slides. The basic method for aCGH is shown in Fig. 18.



**Fig. 18** The aCGH process

## *Gene Cloning*

After predicting gene location, the next step is to predict the function of the gene. One of the approaches is to clone the gene using recombinant DNA approaches (Lesson 5). Tests for gene function may involve in vitro biochemical analyses for the activity of an enzyme, or complementation of a mutant phenotype by the wild type allele. One can use information from comparative analysis of a species with a simple genome to clone genes from a species with a complex genome. For example, the isolation of the R3a blight resistance gene in potato utilized genomic information from tomato (Huang et al., 2005).

### FYI: Examples



**Fig. 19** Blight resistance in the potato plant was aided by genomic information from tomato. Photo by U.S. Department of Agriculture.



## *Analysis of Genome Evolution*

Evolution of a species is a result of numerous processes including gene duplication and loss, whole genome duplication, variation in ploidy level, retrotransposon activity, and genome rearrangements. Genome evolution describes how the genome has been rearranged through time. Thus, to understand the evolution of a species we need to analyze genome evolution. Genome analysis involves construction of a map in one species and comparison of the map with maps from closely related species by the means of common markers (or common single gene traits).

An understanding of crop origins has long been held as central to the identification of useful genetic resources for crop improvement. The number of times that a species has been domesticated influences the genetic architecture of agronomic traits and the levels of genetic diversity in crop genomes. Domestication shapes the genetic variation that is available to modern breeders as it influences levels of nucleotide diversity and patterns of LD (linkage disequilibrium) genome-wide. The demographic history of domestication also informs our expectations of the genetic architecture of traits and thus our ability to identify causal genetic variants for crop improvement.

## *Genome Evolution: Details*

There is evidence for both single domestications (such as maize and soybeans) and multiple domestications (such as avocados, common beans and barley); but for most crops it is not known whether single or multiple domestication events were involved. Following domestication, extensive admixture with wild relatives may occur; and this may be one explanation for the continued controversy regarding the origins of the domesticated indica and japonica rice.

Isolation of genes encoding domestication traits bears evolutionary importance. Until recently, traits that facilitated domestication, i.e. 'domestication syndrome' including decreased dispersal, reduced branching, loss of seed dormancy, reduced natural defenses and increased size of certain morphological features were investigated using mapping strategies. Thus, the study was limited to only a handful traits or loci. Whole-genome data of crops and their wild relatives will facilitate identification of complex demographic histories of many crops. Population genetic approaches, e.g. genome wide association studies (GWAS) will help identify loci that have no known phenotypes; e.g. 2-4% of loci in the maize were affected by artificial selection during domestication. Also, Nextgen sequencing will reveal genome-wide polymorphisms among the accessions leading to discovering demographic history and geographic origins of crop plants.

## Analysis of Genome Evolution

Comparative genetic mapping studies between species suggested some similarity in the genetic basis of domestication syndrome traits (orthology). Comparative genomics studies in both maize and sunflowers suggest selection on genes for amino acid biosynthesis (unknowingly) during domestication contributes to heterosis.



**Fig. 20 Sunflowers in Fargo, North Dakota, USA. Studies suggest that the domestication of sunflowers unknowingly contributes to heterosis. Photo by the U.S. Department of Agriculture.**

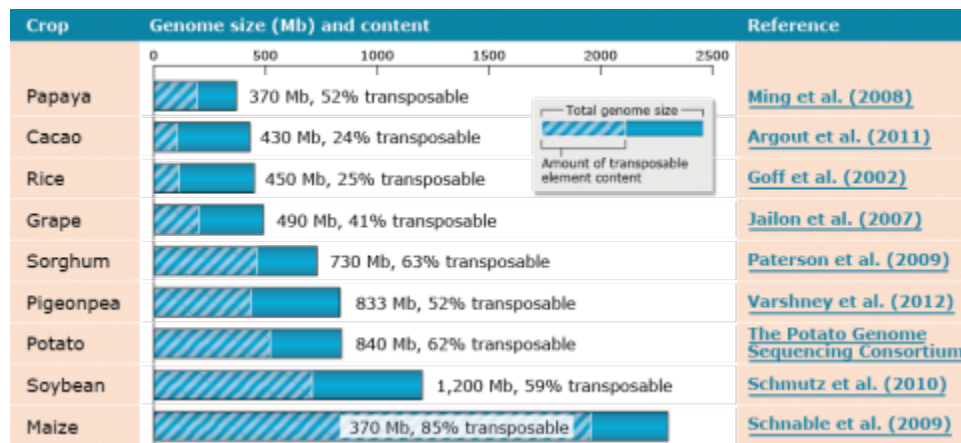
### *Challenges: Large Genomes*

Most genome tools were not developed for plant genomics studies. First generation molecular markers were isozyme markers that were available in the late 1960s for mapping plant genomes. But such markers are limited in number, and DNA markers paved the way towards construction of high-density molecular maps in 1990s.

Despite the availability of DNA markers, large size of plant genomes remains the greatest challenge in plant comparative genomics.

## Challenges: Transposable Content

Large genome sizes for plant species are a result of amplification of retrotransposable elements (Fig. 21). In addition, plants genomes contain multi-gene families and paralogous genes that are tandem-duplicated; for example, plant disease resistance genes.



**Fig. 21 Genome characteristics of common crop species.**

## Challenges: Map Assembly

Duplicated and paralogous sequences, and transposable elements are difficult to assemble during the process of building a genome map (Fig. 22). In Fig. 22 colored shapes represent transposable elements or genes; genes X are a pair of paralogous genes. Short sequence reads are shown directly above where they would map to the reference.

### scenario 1



Fig. 22 The mapping of short sequence reads to a reference plant genome. Adapted from Morrell et al., 2012.

## Challenges: Map Assembly

Duplicated and paralogous sequences, and transposable elements are difficult to assemble during the process of building a genome map (Fig. 23). In Fig. 23 colored shapes represent transposable elements or genes; genes X are a pair of paralogous genes. Short sequence reads are shown directly above where they would map to the reference.

### scenario 2

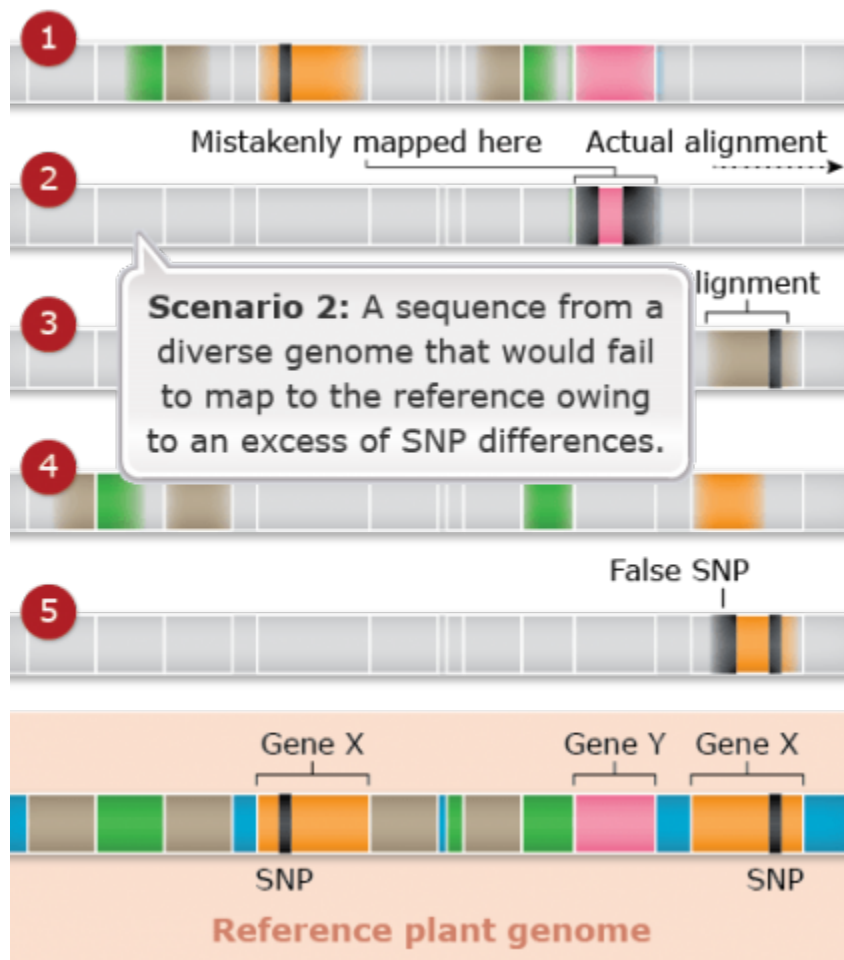


Fig. 23 The mapping of short sequence reads to a reference plant genome. Adapted from Morrell et al., 2012.

## Challenges: Map Assembly

Duplicated and paralogous sequences, and transposable elements are difficult to assemble during the process of building a genome map (Fig. 24). In Fig. 24 colored shapes represent transposable elements or genes; genes X are a pair of paralogous genes. Short sequence reads are shown directly above where they would map to the reference.

### scenario 3

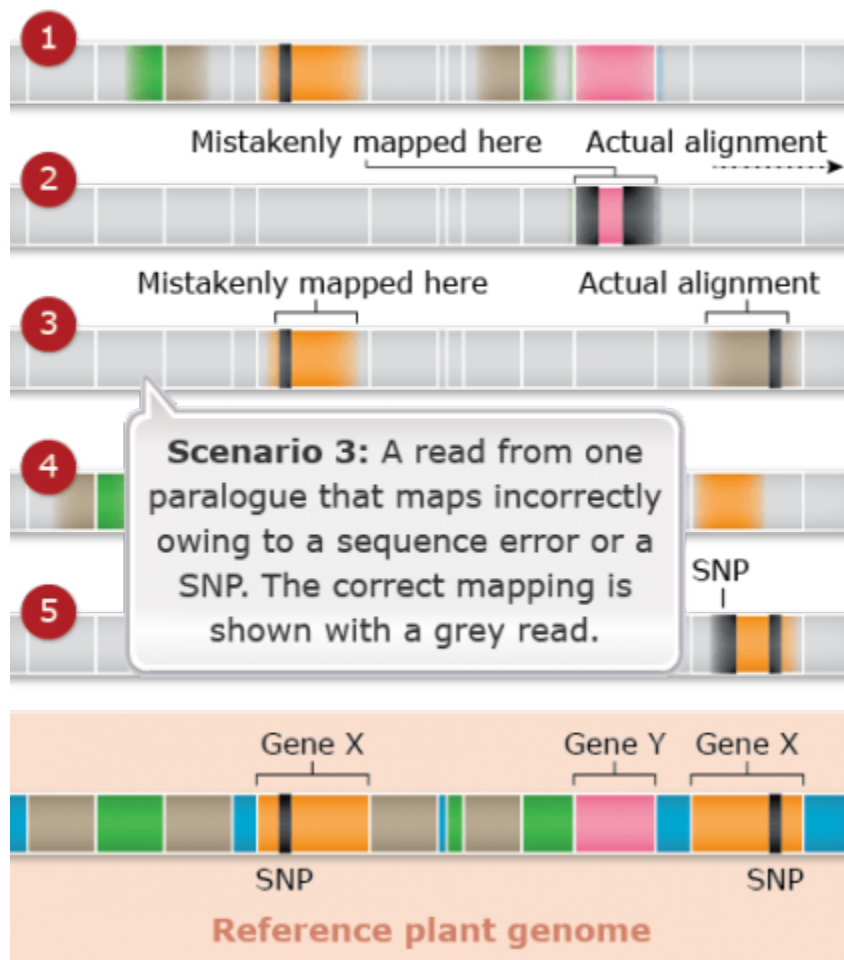


Fig. 24 The mapping of short sequence reads to a reference plant genome. Adapted from Morrell et al., 2012.



## Challenges: Map Assembly

Duplicated and paralogous sequences, and transposable elements are difficult to assemble during the process of building a genome map (Fig. 25). In Fig. 25 colored shapes represent transposable elements or genes; genes X are a pair of paralogous genes. Short sequence reads are shown directly above where they would map to the reference.

### scenario 4

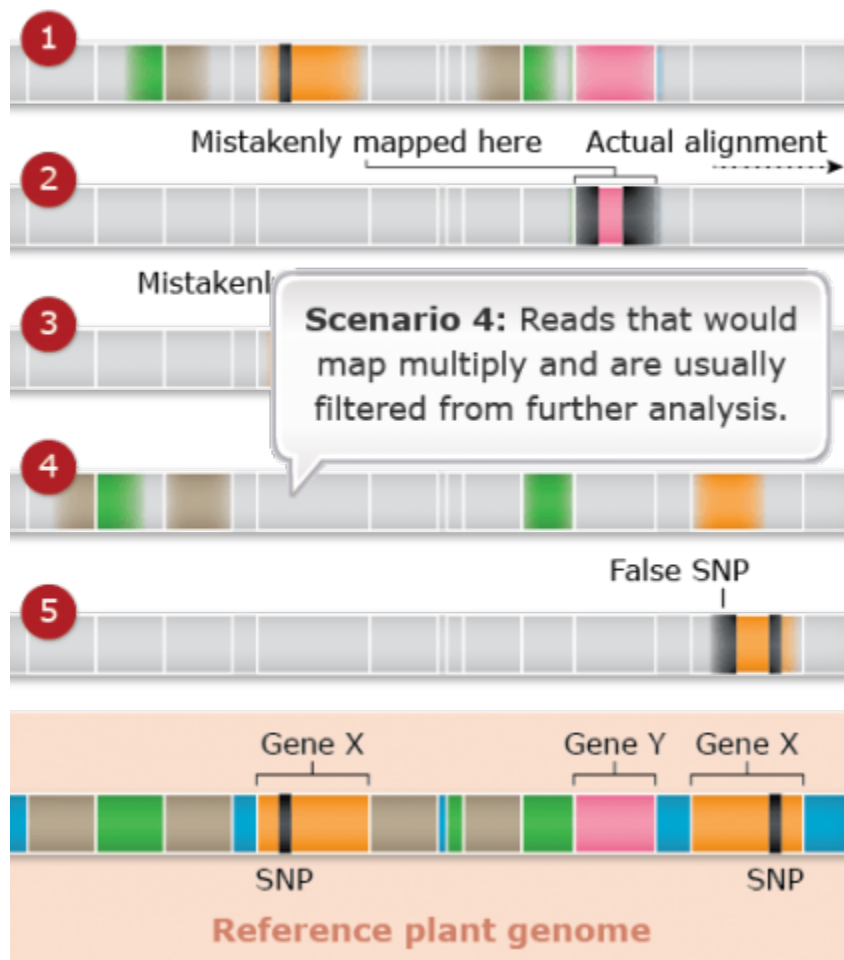


Fig. 25 The mapping of short sequence reads to a reference plant genome. Adapted from Morrell et al., 2012.

## Challenges: Map Assembly

Duplicated and paralogous sequences, and transposable elements are difficult to assemble during the process of building a genome map (Fig. 26). In Fig. 26 colored shapes represent transposable elements or genes; genes X are a pair of paralogous genes. Short sequence reads are shown directly above where they would map to the reference.

### scenario 5

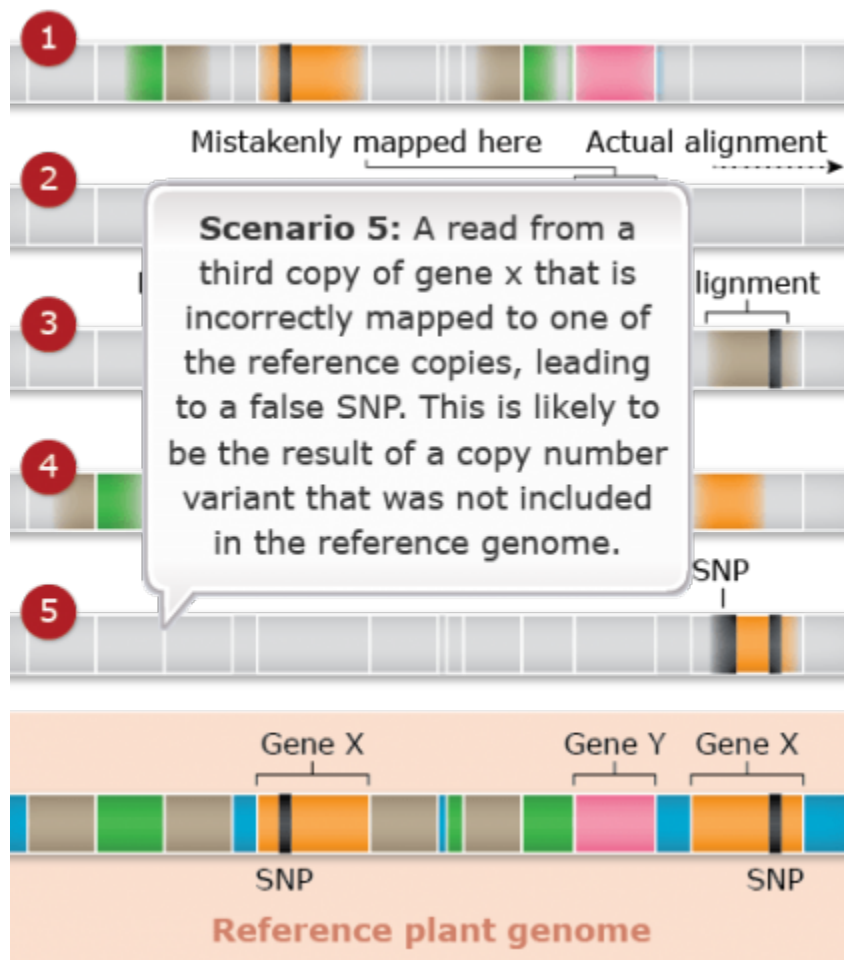


Fig. 26 The mapping of short sequence reads to a reference plant genome. Adapted from Morrell et al., 2012.

### *Challenges: Repeated Sequences*

High proportion of repeated sequences also makes it difficult to conduct reference genome-based SNP identification and genome-wide association studies. Therefore, regardless of the emerging high throughput sequencing technologies, it remains challenging to achieve sufficient genome coverage for assembling short read sequences and paralogous sequences. Consequently, fewer crop species with large genomes have been sequenced so far. Improvement in sequence read length by nextgen approaches will reduce this problem allowing detection of local patterns of LD for identifying paralogous reads in complex crop genomes.



**Fig. 27** A pigeonpea plant in Ayotupas, West Timor, Indonesia. The pigeonpea genome is among those whose sequence has been published. Photo by Wibawo Djatmiko. Liscensed under CC BY-SA 3.0 via Wikimedia Commons.

## Summary

Comparative genomics is a field of research focusing on determining the evolutionary relationships of genomes and link differences to functional consequences, or phenotypes. With progress in sequencing technology, an increased number of plant genomes have been sequenced making it possible to construct comparative maps and predict gene pairs of two species. To understand how genomes evolve, a genome map is constructed in one species and compared with maps from closely related species by the means of common markers. The majority of the comparative mapping studies are based on conservation of nucleotide sequences among closely related species. Comparative genomics is also useful for the identification of genes. Following prediction of gene location by comparative analysis, target genes may be isolated and characterized to determine their function. However, one of the greatest challenges in plant comparative genomics is the large size of plant genomes. Consequently, fewer crop species with large genomes have currently been sequenced.

## Reflection

The **Module Reflection** appears as the last "task" in each module. The purpose of the Reflection is to enhance your learning and information retention. The questions are designed to help you reflect on the module and obtain instructor feedback on your learning. Submit your answers to the following questions to your instructor.

1. In your own words, write a short summary (< 150 words) for this module.
2. What is the most valuable concept that you learned from the module?
3. Why is this concept valuable to you? What concepts in the module are still unclear/the least clear to you?

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## For Your Information

### *FYI: aCGH Applications*

Examples of aCGH application in plant research:

[>>](#) Comparative Genomics of Host-Specific Virulence in *Pseudomonas syringae*

[>>](#) A Microarray Based Genomic Hybridization Method for Identification of New Genes in Plants: Case Analyses of *Arabidopsis* and *Oryza*

## *FYI: Cloning Approaches*

Plant gene cloning by comparative genomic approaches

>> Comparative genomics enabled the isolation of the R3a late blight resistance gene in potato

>> Plant gene cloning may lead to better timing of flowering

# Acknowledgements

This module was developed as part of the Bill & Melinda Gates Foundation Contract No. 24576 for Plant Breeding E-Learning in Africa.

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**How to cite this module:** Bhattacharyya, M. and W. Suza. 2016. Comparative Mapping and Genomics. *In* Molecular Plant Breeding, interactive e-learning courseware. Plant Breeding E-Learning in Africa. Retrieved from <https://pbea.agron.iastate.edu>.

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