

# Bioinformatics Toolbox™ 3

## User's Guide

MATLAB®

## How to Contact MathWorks



[www.mathworks.com](http://www.mathworks.com) Web  
[comp.soft-sys.matlab](mailto:comp.soft-sys.matlab) Newsgroup  
[www.mathworks.com/contact\\_TS.html](http://www.mathworks.com/contact_TS.html) Technical Support



[suggest@mathworks.com](mailto:suggest@mathworks.com) Product enhancement suggestions  
[bugs@mathworks.com](mailto:bugs@mathworks.com) Bug reports  
[doc@mathworks.com](mailto:doc@mathworks.com) Documentation error reports  
[service@mathworks.com](mailto:service@mathworks.com) Order status, license renewals, passcodes  
[info@mathworks.com](mailto:info@mathworks.com) Sales, pricing, and general information



508-647-7000 (Phone)



508-647-7001 (Fax)



The MathWorks, Inc.  
3 Apple Hill Drive  
Natick, MA 01760-2098

For contact information about worldwide offices, see the MathWorks Web site.

*Bioinformatics Toolbox™ User's Guide*

© COPYRIGHT 2003–2011 by The MathWorks, Inc.

The software described in this document is furnished under a license agreement. The software may be used or copied only under the terms of the license agreement. No part of this manual may be photocopied or reproduced in any form without prior written consent from The MathWorks, Inc.

FEDERAL ACQUISITION: This provision applies to all acquisitions of the Program and Documentation by, for, or through the federal government of the United States. By accepting delivery of the Program or Documentation, the government hereby agrees that this software or documentation qualifies as commercial computer software or commercial computer software documentation as such terms are used or defined in FAR 12.212, DFARS Part 227.72, and DFARS 252.227-7014. Accordingly, the terms and conditions of this Agreement and only those rights specified in this Agreement, shall pertain to and govern the use, modification, reproduction, release, performance, display, and disclosure of the Program and Documentation by the federal government (or other entity acquiring for or through the federal government) and shall supersede any conflicting contractual terms or conditions. If this License fails to meet the government's needs or is inconsistent in any respect with federal procurement law, the government agrees to return the Program and Documentation, unused, to The MathWorks, Inc.

### Trademarks

MATLAB and Simulink are registered trademarks of The MathWorks, Inc. See [www.mathworks.com/trademarks](http://www.mathworks.com/trademarks) for a list of additional trademarks. Other product or brand names may be trademarks or registered trademarks of their respective holders.

### Patents

MathWorks products are protected by one or more U.S. patents. Please see [www.mathworks.com/patents](http://www.mathworks.com/patents) for more information.

## Revision History

September 2003	Online only	New for Version 1.0 (Release 13SP1+)
June 2004	Online only	Revised for Version 1.1 (Release 14)
November 2004	Online only	Revised for Version 2.0 (Release 14SP1+)
March 2005	Online only	Revised for Version 2.0.1 (Release 14SP2)
May 2005	Online only	Revised for Version 2.1 (Release 14SP2+)
September 2005	Online only	Revised for Version 2.1.1 (Release 14SP3)
November 2005	Online only	Revised for Version 2.2 (Release 14SP3+)
March 2006	Online only	Revised for Version 2.2.1 (Release 2006a)
May 2006	Online only	Revised for Version 2.3 (Release 2006a+)
September 2006	Online only	Revised for Version 2.4 (Release 2006b)
March 2007	Online only	Revised for Version 2.5 (Release 2007a)
April 2007	Online only	Revised for Version 2.6 (Release 2007a+)
September 2007	Online only	Revised for Version 3.0 (Release 2007b)
March 2008	Online only	Revised for Version 3.1 (Release 2008a)
October 2008	Online only	Revised for Version 3.2 (Release 2008b)
March 2009	Online only	Revised for Version 3.3 (Release 2009a)
September 2009	Online only	Revised for Version 3.4 (Release 2009b)
March 2010	Online only	Revised for Version 3.5 (Release 2010a)
September 2010	Online only	Revised for Version 3.6 (Release 2010b)
April 2011	Online only	Revised for Version 3.7 (Release 2011a)



## Getting Started

**1**

<b>Product Overview</b> .....	1-2
Features .....	1-2
Expected Users .....	1-3
<b>Installation</b> .....	1-5
Installing .....	1-5
Required Software .....	1-5
Optional Software .....	1-6
<b>Features and Functions</b> .....	1-8
Data Formats and Databases .....	1-8
Sequence Alignments .....	1-10
Sequence Utilities and Statistics .....	1-10
Protein Property Analysis .....	1-11
Phylogenetic Analysis .....	1-12
Microarray Data Analysis .....	1-12
Microarray Data Storage .....	1-13
Mass Spectrometry Data Analysis .....	1-14
Graph Theory Functions .....	1-17
Graph Visualization .....	1-18
Statistical Learning and Visualization .....	1-18
Prototyping and Development Environment .....	1-19
Data Visualization .....	1-19
Algorithm Sharing and Application Deployment .....	1-20
<b>Importing and Exploring Bioinformatic Data from     Microsoft® Excel</b> .....	1-21
<b>Creating get Functions</b> .....	1-29
What Are get Functions? .....	1-29
Creating the getpubmed Function .....	1-30

<b>Extracting Information from Large Multi-Entry Text</b>	
<b>Files</b> .....	1-34
Overview .....	1-34
What Files Can You Access? .....	1-34
Before You Begin .....	1-35
Creating a BioIndexedFile Object to Access Your Source	
File .....	1-36
Determining the Number of Entries Indexed By a	
BioIndexedFile Object .....	1-37
Retrieving Entries from Your Source File .....	1-37
Reading Entries from Your Source File .....	1-38

## Sequence Analysis

# 2

<b>Example: Sequence Statistics</b> .....	2-2
Overview of Example .....	2-2
Determining Nucleotide Content .....	2-2
Reading Sequence Information .....	2-5
Determining Nucleotide Composition .....	2-6
Determining Codon Composition .....	2-11
Open Reading Frames .....	2-15
Amino Acid Conversion and Composition .....	2-18
 <b>Example: Sequence Alignment</b> .....	2-22
Overview of Example .....	2-22
Finding a Model Organism to Study .....	2-22
Retrieving Sequence Information from a Public	
Database .....	2-24
Searching a Public Database for Related Genes .....	2-27
Locating Protein Coding Sequences .....	2-29
Comparing Amino Acid Sequences .....	2-33
 <b>Sequence Tool</b> .....	2-42
Overview of the Sequence Tool .....	2-42
Importing a Sequence .....	2-42
Viewing Nucleotide Sequence Information .....	2-44
Searching for Words .....	2-46
Exploring Open Reading Frames .....	2-48
Viewing Amino Acid Sequence Statistics .....	2-51

Closing the Sequence Tool .....	2-55
References .....	2-55
<b>Multiple Sequence Alignment Viewer .....</b>	<b>2-56</b>
Overview of the Multiple Sequence Alignment Viewer ...	2-56
Loading Sequence Data and Viewing the Phylogenetic Tree .....	2-56
Selecting a Subset of Data from the Phylogenetic Tree ...	2-57
Aligning Multiple Sequences .....	2-58
Adjusting Multiple Sequence Alignments Manually .....	2-59
Closing the Multiple Sequence Alignment Viewer .....	2-62
<b>Storing and Managing Short-Read Sequence Data in Objects .....</b>	<b>2-63</b>
Overview .....	2-63
Representing Sequence and Quality Data in a BioRead Object .....	2-64
Representing Sequence, Quality, and Alignment/Mapping Data in a BioMap Object .....	2-66
Retrieving Information from a BioRead or BioMap Object .....	2-70
Setting Information in a BioRead or BioMap Object .....	2-72
Determining Coverage of a Reference Sequence .....	2-73
Constructing Sequence Alignments to a Reference Sequence .....	2-75
Filtering Read Sequences Using SAM Flags .....	2-76

## Microarray Analysis

### 3

<b>Storing and Managing Gene Expression Data in Objects .....</b>	<b>3-2</b>
<b>Representing Expression Data Values in DataMatrix Objects .....</b>	<b>3-5</b>
Overview of DataMatrix Objects .....	3-5
Constructing DataMatrix Objects .....	3-6
Getting and Setting Properties of a DataMatrix Object ...	3-7
Accessing Data in DataMatrix Objects .....	3-8

<b>Representing Expression Data Values in ExptData</b>	
<b>Objects</b> .....	3-11
Overview of ExptData Objects .....	3-11
Constructing ExptData Objects .....	3-12
Using Properties of an ExptData Object .....	3-12
Using Methods of an ExptData Object .....	3-13
References .....	3-14
<b>Representing Sample and Feature Metadata in</b>	
<b>MetaData Objects</b> .....	3-15
Overview of MetaData Objects .....	3-15
Constructing MetaData Objects .....	3-16
Using Properties of a MetaData Object .....	3-19
Using Methods of a MetaData Object .....	3-20
<b>Representing Experiment Information in a MIAME</b>	
<b>Object</b> .....	3-22
Overview of MIAME Objects .....	3-22
Constructing MIAME Objects .....	3-22
Using Properties of a MIAME Object .....	3-25
Using Methods of a MIAME Object .....	3-25
<b>Representing All Data and Information in an</b>	
<b>ExpressionSet Object</b> .....	3-27
Overview of ExpressionSet Objects .....	3-27
Constructing ExpressionSet Objects .....	3-29
Using Properties of an ExpressionSet Object .....	3-30
Using Methods of an ExpressionSet Object .....	3-30
<b>Example: Visualizing Microarray Data</b> .....	3-33
Overview of the Mouse Example .....	3-33
Exploring the Microarray Data Set .....	3-34
Spatial Images of Microarray Data .....	3-36
Statistics of the Microarrays .....	3-46
Scatter Plots of Microarray Data .....	3-48
<b>Example: Analyzing Gene Expression Profiles</b> .....	3-57
Overview of the Yeast Example .....	3-57
Exploring the Data Set .....	3-57
Filtering Genes .....	3-61
Clustering Genes .....	3-64
Principal Component Analysis .....	3-68



4

**Overview of Phylogenetic Analysis** ..... 4-2

**Example: Building a Phylogenetic Tree** ..... 4-3

    Overview of the Primate Example ..... 4-3

    Searching NCBI for Phylogenetic Data ..... 4-5

    Creating a Phylogenetic Tree for Five Species ..... 4-6

    Creating a Phylogenetic Tree for Twelve Species ..... 4-9

    Exploring the Phylogenetic Tree ..... 4-11

**Phylogenetic Tree Tool Reference** ..... 4-16

    Overview of the Phylogenetic Tree Tool ..... 4-16

    Opening the Phylogenetic Tree Tool ..... 4-16

    File Menu ..... 4-18

    Tools Menu ..... 4-31

    Window Menu ..... 4-40

    Help Menu ..... 4-40

Examples

A

**Introduction** ..... A-2

**Sequence Analysis** ..... A-2

**Microarray Analysis** ..... A-2

**Phylogenetic Analysis** ..... A-2

Index



# Getting Started

---

- “Product Overview” on page 1-2
- “Installation” on page 1-5
- “Features and Functions” on page 1-8
- “Importing and Exploring Bioinformatic Data from Microsoft® Excel” on page 1-21
- “Creating get Functions” on page 1-29
- “Extracting Information from Large Multi-Entry Text Files” on page 1-34

## Product Overview

In this section...
“Features” on page 1-2
“Expected Users” on page 1-3

### Features

The Bioinformatics Toolbox™ product extends the MATLAB® environment to provide an integrated software environment for genome and proteome analysis. Scientists and engineers can answer questions, solve problems, prototype new algorithms, and build applications for drug discovery and design, genetic engineering, and biological research. An introduction to these features will help you to develop a conceptual model for working with the toolbox and your biological data.

The Bioinformatics Toolbox product includes many functions to help you with genome and proteome analysis. Most functions are implemented in the MATLAB programming language, with the source available for you to view. This open environment lets you explore and customize the existing toolbox algorithms or develop your own.

You can use the basic bioinformatic functions provided with this toolbox to create more complex algorithms and applications. These robust and well-tested functions are the functions that you would otherwise have to create yourself.

Toolbox features and functions fall within these categories:

- **Data formats and databases** — Connect to Web-accessible databases containing genomic and proteomic data. Read and convert between multiple data formats.
- **Sequence analysis** — Determine the statistical characteristics of a sequence, align two sequences, and multiply align several sequences. Model patterns in biological sequences using hidden Markov model (HMM) profiles.
- **Phylogenetic analysis** — Create and manipulate phylogenetic tree data.

- **Microarray data analysis** — Read, normalize, and visualize microarray data.
- **Mass spectrometry data analysis** — Analyze and enhance raw mass spectrometry data.
- **Statistical learning** — Classify and identify features in data sets with statistical learning tools.
- **Programming interface** — Use other bioinformatic software (BioPerl and BioJava) within the MATLAB environment.

The field of bioinformatics is rapidly growing and will become increasingly important as biology becomes a more analytical science. The toolbox provides an open environment that you can customize for development and deployment of the analytical tools you will need.

- **Prototype and develop algorithms** — Prototype new ideas in an open and extensible environment. Develop algorithms using efficient string processing and statistical functions, view the source code for existing functions, and use the code as a template for customizing, improving, or creating your own functions. See “Prototyping and Development Environment” on page 1-19.
- **Visualize data** — Visualize sequences and alignments, gene expression data, phylogenetic trees, mass spectrometry data, protein structure, and relationships between data with interconnected graphs. See “Data Visualization” on page 1-19.
- **Share and deploy applications** — Use an interactive GUI builder to develop a custom graphical front end for your data analysis programs. Create standalone applications that run separately from the MATLAB environment. See “Algorithm Sharing and Application Deployment” on page 1-20.

## Expected Users

The Bioinformatics Toolbox product is intended for computational biologists and research scientists who need to develop new algorithms or implement published ones, visualize results, and create standalone applications.

- **Industry/Professional** — Increasingly, drug discovery methods are being supported by engineering practice. This toolbox supports tool builders

who want to create applications for the biotechnology and pharmaceutical industries.

- **Education/Professor/Student** — This toolbox is well suited for learning and teaching genome and proteome analysis techniques. Educators and students can concentrate on bioinformatic algorithms instead of programming basic functions such as reading and writing to files.

While the toolbox includes many bioinformatic functions, it is not intended to be a complete set of tools for scientists to analyze their biological data. However, the MATLAB environment is ideal for rapidly designing and prototyping the tools you need.

# Installation

In this section...
“Installing” on page 1-5
“Required Software” on page 1-5
“Optional Software” on page 1-6

## Installing

Install the Bioinformatics Toolbox software from a DVD or Web release using the MathWorks® Installer. For more information, see the installation documentation.

## Required Software

The Bioinformatics Toolbox software requires the following MathWorks products to be installed on your computer.

Required Software	Description
<b>MATLAB</b>	Provides a command-line interface and integrated software environment for the Bioinformatics Toolbox software.  Version 3.7 of the Bioinformatics Toolbox software requires MATLAB Version 7.12 on the Release 2011a DVD.
<b>Statistics Toolbox™</b>	Provides basic statistics and probability functions used by the functions of the Bioinformatics Toolbox software.  Version 3.7 of the Bioinformatics Toolbox software requires Statistics Toolbox Version 7.5 on the Release 2011a DVD.

## Optional Software

MATLAB and the Bioinformatics Toolbox software environment is open and extensible. In this environment you can interactively explore ideas, prototype new algorithms, and develop complete solutions to problems in bioinformatics. MATLAB facilitates computation, visualization, prototyping, and deployment.

Using the Bioinformatics Toolbox software with other MATLAB toolboxes and products will allow you to do advanced algorithm development and solve multidisciplinary problems.

Optional Software	Description
<b>Parallel Computing Toolbox™</b>	Perform parallel bioinformatic computations on multicore computers and computer clusters. For an example of batch processing through parallel computing, see the Batch Processing of Spectra Using Distributed Computing demo.
<b>Signal Processing Toolbox™</b>	Process signal data from bioanalytical instrumentation. Examples include acquisition of fluorescence data for DNA sequence analyzers, fluorescence data for microarray scanners, and mass spectrometric data from protein analyses.
<b>Image Processing Toolbox™</b>	Create complex and custom image processing algorithms for data from microarray scanners.
<b>SimBiology®</b>	Model, simulate, and analyze biochemical systems.
<b>Optimization Toolbox™</b>	Use nonlinear optimization to predict the secondary structure of proteins and the structure of other biological macromolecules.
<b>Neural Network Toolbox™</b>	Use neural networks to solve problems where algorithms are not available. For example, you can train neural networks for pattern recognition using large sets of sequence data.
<b>Database Toolbox™</b>	Create your own in-house databases for sequence data with custom annotations.



<b>Optional Software</b>	<b>Description</b>
<b>MATLAB® Compiler™</b>	Create standalone applications from MATLAB GUI applications, and create dynamic link libraries from MATLAB functions to use with any programming environment.
<b>MATLAB® Builder™ NE</b>	Create COM objects to use with any COM-based programming environment.
<b>MATLAB Builder JA</b>	Integrate MATLAB applications into your organization's Java™ programs by creating a Java wrapper around the application.
<b>MATLAB Builder EX</b>	Create Microsoft® Excel® add-in functions from MATLAB functions to use with Excel® spreadsheets.
<b>Spreadsheet Link™ EX</b>	Connect Microsoft Excel with the MATLAB Workspace to exchange data and to use MATLAB computational and visualization functions. For more information, see “Importing and Exploring Bioinformatic Data from Microsoft® Excel” on page 1-21.

## Features and Functions

### In this section...

“Data Formats and Databases” on page 1-8

“Sequence Alignments” on page 1-10

“Sequence Utilities and Statistics” on page 1-10

“Protein Property Analysis” on page 1-11

“Phylogenetic Analysis” on page 1-12

“Microarray Data Analysis” on page 1-12

“Microarray Data Storage” on page 1-13

“Mass Spectrometry Data Analysis” on page 1-14

“Graph Theory Functions” on page 1-17

“Graph Visualization” on page 1-18

“Statistical Learning and Visualization” on page 1-18

“Prototyping and Development Environment” on page 1-19

“Data Visualization” on page 1-19

“Algorithm Sharing and Application Deployment” on page 1-20

### Data Formats and Databases

The toolbox accesses many of the databases on the Web and other online data sources. It allows you to copy data into the MATLAB Workspace, and read and write to files with standard bioinformatic formats. It also reads many common genome file formats, so that you do not have to write and maintain your own file readers.

**Web-based databases** — You can directly access public databases on the Web and copy sequence and gene expression information into the MATLAB environment.

The sequence databases currently supported are GenBank® (`getgenbank`), GenPept (`getgenpept`), European Molecular Biology Laboratory (EMBL) (`getembl`), and Protein Data Bank (PDB) (`getpdb`). You can also access data

from the NCBI Gene Expression Omnibus (GEO) Web site by using a single function (`getgeodata`).

Get multiply aligned sequences (`gethmmalignment`), hidden Markov model profiles (`gethmmprof`), and phylogenetic tree data (`gethmmtree`) from the PFAM database.

**Gene Ontology database** — Load the database from the Web into a gene ontology object (`geneont.geneont`). Select sections of the ontology with methods for the `geneont` object (`geneont.getancestors`, `geneont.getdescendants`, `geneont.getmatrix`, `geneont.getrelatives`), and manipulate data with utility functions (`goannotread`, `num2goid`).

**Read data from instruments** — Read data generated from gene sequencing instruments (`scfread`, `joinseq`, `traceplot`), mass spectrometers (`jcampread`), and Agilent<sup>®</sup> microarray scanners (`agferead`).

**Reading data formats** — The toolbox provides a number of functions for reading data from common bioinformatic file formats.

- Sequence data: GenBank (`genbankread`), GenPept (`genpeptread`), EMBL (`emblread`), PDB (`pdbread`), and FASTA (`fastaread`)
- Multiply aligned sequences: ClustalW and GCG formats (`multialignread`)
- Gene expression data from microarrays: Gene Expression Omnibus (GEO) data (`geosoftread`), GenePix<sup>®</sup> data in GPR and GAL files (`gprread`, `galread`), SPOT data (`sptread`), Affymetrix<sup>®</sup> GeneChip<sup>®</sup> data (`affyread`), and Imagen<sup>®</sup> results files (`imageneread`)
- Hidden Markov model profiles: PFAM-HMM file (`pfamhmmread`)

**Writing data formats** — The functions for getting data from the Web include the option to save the data to a file. However, there is a function to write data to a file using the FASTA format (`fastawrite`).

**BLAST searches** — Request Web-based BLAST searches (`blastncbi`), get the results from a search (`getblast`) and read results from a previously saved BLAST formatted report file (`blastread`).

The MATLAB environment has built-in support for other industry-standard file formats including Microsoft Excel and comma-separated-value (CSV) files. Additional functions perform ASCII and low-level binary I/O, allowing you to develop custom functions for working with any data format.

## Sequence Alignments

You can select from a list of analysis methods to compare nucleotide or amino acid sequences using pairwise or multiple sequence alignment functions.

**Pairwise sequence alignment** — Efficient implementations of standard algorithms such as the Needleman-Wunsch (`nwalign`) and Smith-Waterman (`swalign`) algorithms for pairwise sequence alignment. The toolbox also includes standard scoring matrices such as the PAM and BLOSUM families of matrices (`blosum`, `dayhoff`, `gonnet`, `nuc44`, `pam`). Visualize sequence similarities with `seqdotplot` and sequence alignment results with `showalignment`.

**Multiple sequence alignment** — Functions for multiple sequence alignment (`multialign`, `profalign`) and functions that support multiple sequences (`multialignread`, `fastaread`, `showalignment`). There is also a graphical interface (`multialignviewer`) for viewing the results of a multiple sequence alignment and manually making adjustment.

**Multiple sequence profiles** — Implementations for multiple alignment and profile hidden Markov model algorithms (`gethmmprof`, `gethmmalignment`, `gethmmtree`, `pfamhmmread`, `hmmprofalign`, `hmmprofestimate`, `hmmprofgenerate`, `hmmprofmerge`, `hmmprofstruct`, `showhmmprof`).

**Biological codes** — Look up the letters or numeric equivalents for commonly used biological codes (`aminolookup`, `baselookup`, `geneticcode`, `revgeneticcode`).

## Sequence Utilities and Statistics

You can manipulate and analyze your sequences to gain a deeper understanding of the physical, chemical, and biological characteristics of your data. Use a graphical user interface (GUI) with many of the sequence functions in the toolbox (`seqtool`).

**Sequence conversion and manipulation** — The toolbox provides routines for common operations, such as converting DNA or RNA sequences to amino acid sequences, that are basic to working with nucleic acid and protein sequences (`aa2int`, `aa2nt`, `dna2rna`, `rna2dna`, `int2aa`, `int2nt`, `nt2aa`, `nt2int`, `seqcomplement`, `seqrcomplement`, `seqreverse`).

You can manipulate your sequence by performing an *in silico* digestion with restriction endonucleases (`restrict`) and proteases (`cleave`).

**Sequence statistics** — Determine various statistics about a sequence (`aacount`, `basecount`, `codoncount`, `dimercount`, `nmercount`, `ntdensity`, `codonbias`, `cpgisland`, `oligoprop`), search for specific patterns within a sequence (`seqshowwords`, `seqwordcount`), or search for open reading frames (`seqshoworfs`). In addition, you can create random sequences for test cases (`randseq`).

**Sequence utilities** — Determine a consensus sequence from a set of multiply aligned amino acid, nucleotide sequences (`seqconsensus`, or a sequence profile (`seqprofile`)). Format a sequence for display (`seqdisp`) or graphically show a sequence alignment with frequency data (`seqlogo`).

Additional MATLAB functions efficiently handle string operations with regular expressions (`regexp`, `seq2regexp`) to look for specific patterns in a sequence and search through a library for string matches (`seqmatch`).

Look for possible cleavage sites in a DNA/RNA sequence by searching for palindromes (`palindromes`).

## Protein Property Analysis

You can use a collection of protein analysis methods to extract information from your data. You can determine protein characteristics and simulate enzyme cleavage reactions. The toolbox provides functions to calculate various properties of a protein sequence, such as the atomic composition (`atomiccomp`), molecular weight (`molweight`), and isoelectric point (`isoelectric`). You can cleave a protein with an enzyme (`cleave`, `rebasecuts`) and create distance and Ramachandran plots for PDB data (`pdbdistplot`, `ramachandran`). The toolbox contains a graphical user interface for protein analysis (`proteinplot`) and plotting 3-D protein and other molecular structures with information from molecule model files, such as PDB files (`molviewer`).

**Amino acid sequence utilities** — Calculate amino acid statistics for a sequence (`aaccount`) and get information about character codes (`aminolookup`).

## Phylogenetic Analysis

You can use functions for phylogenetic tree building and analysis. There is also a GUI to draw phylograms (`trees`).

**Phylogenetic tree data** — Read and write Newick-formatted tree files (`phytreeread`, `phytreewrite`) into the MATLAB Workspace as phylogenetic tree objects (`phytree`).

**Create a phylogenetic tree** — Calculate the pairwise distance between biological sequences (`seqpdist`), estimate the substitution rates (`dnds`, `dndsml`), build a phylogenetic tree from pairwise distances (`seqlinkage`, `seqneighjoin`, `reroot`), and view the tree in an interactive GUI that allows you to view, edit, and explore the data (`phytreetool` or `view`). This GUI also allows you to prune branches, reorder, rename, and explore distances.

**Phylogenetic tree object methods** — You can access the functionality of the `phytreetool` GUI using methods for a phylogenetic tree object (`phytree`). Get property values (`get`) and node names (`getbyname`). Calculate the patristic distances between pairs of leaf nodes (`pdist`, `weights`) and draw a phylogenetic tree object in a MATLAB Figure window as a phylogram, cladogram, or radial treeplot (`plot`). Manipulate tree data by selecting branches and leaves using a specified criterion (`select`, `subtree`) and removing nodes (`prune`). Compare trees (`getcanonical`) and use Newick-formatted strings (`getnewickstr`).

## Microarray Data Analysis

The MATLAB environment is widely used for microarray data analysis, including reading, filtering, normalizing, and visualizing microarray data. However, the standard normalization and visualization tools that scientists use can be difficult to implement. The toolbox includes these standard functions:

**Microarray data** — Read Affymetrix GeneChip files (`affyread`) and plot data (`probesetplot`), ImaGene results files (`imageneread`), SPOT files (`sptread`) and Agilent microarray scanner files (`agferead`). Read GenePix

GPR files (`gprread`) and GAL files (`galread`). Get Gene Expression Omnibus (GEO) data from the Web (`getgeodata`) and read GEO data from files (`geosoftread`).

A utility function (`magetfield`) extracts data from one of the microarray reader functions (`gprread`, `agferead`, `sptread`, `imageneread`).

**Microarray normalization and filtering** — The toolbox provides a number of methods for normalizing microarray data, such as lowest normalization (`malowess`) and mean normalization (`manorm`), or across multiple arrays (`quantilenorm`). You can use filtering functions to clean raw data before analysis (`geneentropyfilter`, `genelowvalfilter`, `generangefilter`, `genevarfilter`), and calculate the range and variance of values (`exprprofrange`, `exprprofvar`).

**Microarray visualization** — The toolbox contains routines for visualizing microarray data. These routines include spatial plots of microarray data (`maimage`, `redgreenmap`), box plots (`maboxplot`), loglog plots (`maloglog`), and intensity-ratio plots (`mairplot`). You can also view clustered expression profiles (`clustergram`, `redgreenmap`). You can create 2-D scatter plots of principal components from the microarray data (`mapcaplot`).

**Microarray utility functions** — Use the following functions to work with Affymetrix GeneChip data sets. Get library information for a probe (`probelibraryinfo`), gene information from a probe set (`probesetlookup`), and probe set values from CEL and CDF information (`probesetvalues`). Show probe set information from NetAffx™ Analysis Center (`probesetlink`) and plot probe set values (`probesetplot`).

The toolbox accesses statistical routines to perform cluster analysis and to visualize the results, and you can view your data through statistical visualizations such as dendrograms, classification, and regression trees.

## Microarray Data Storage

The toolbox includes functions, objects, and methods for creating, storing, and accessing microarray data.

The object constructor function, `DataMatrix`, lets you create a `DataMatrix` object to encapsulate data and metadata from a microarray experiment. A

DataMatrix object stores experimental data in a matrix, with rows typically corresponding to gene names or probe identifiers, and columns typically corresponding to sample identifiers. A DataMatrix object also stores metadata, including the gene names or probe identifiers (as the row names) and sample identifiers (as the column names).

You can reference microarray expression values in a DataMatrix object the same way you reference data in a MATLAB array, that is, by using linear or logical indexing. Alternately, you can reference this experimental data by gene (probe) identifiers and sample identifiers. Indexing by these identifiers lets you quickly and conveniently access subsets of the data without having to maintain additional index arrays.

Many MATLAB operators and arithmetic functions are available to DataMatrix objects by means of methods. These methods let you modify, combine, compare, analyze, plot, and access information from DataMatrix objects. Additionally, you can easily extend the functionality by using general element-wise functions, `dmarrayfun` and `dmbsxfun`, and by manually accessing the properties of a DataMatrix object.

---

**Note** For more information on creating and using DataMatrix objects, see “Representing Expression Data Values in DataMatrix Objects” on page 3-5.

---

## **Mass Spectrometry Data Analysis**

The mass spectrometry functions preprocess and classify raw data from SELDI-TOF and MALDI-TOF spectrometers and use statistical learning functions to identify patterns.

**Reading raw data** — Load raw mass/charge and ion intensity data from comma-separated-value (CSV) files, or read a JCAMP-DX-formatted file with mass spectrometry data (`jcampread`) into the MATLAB environment.

You can also have data in TXT files and use the `importdata` function.

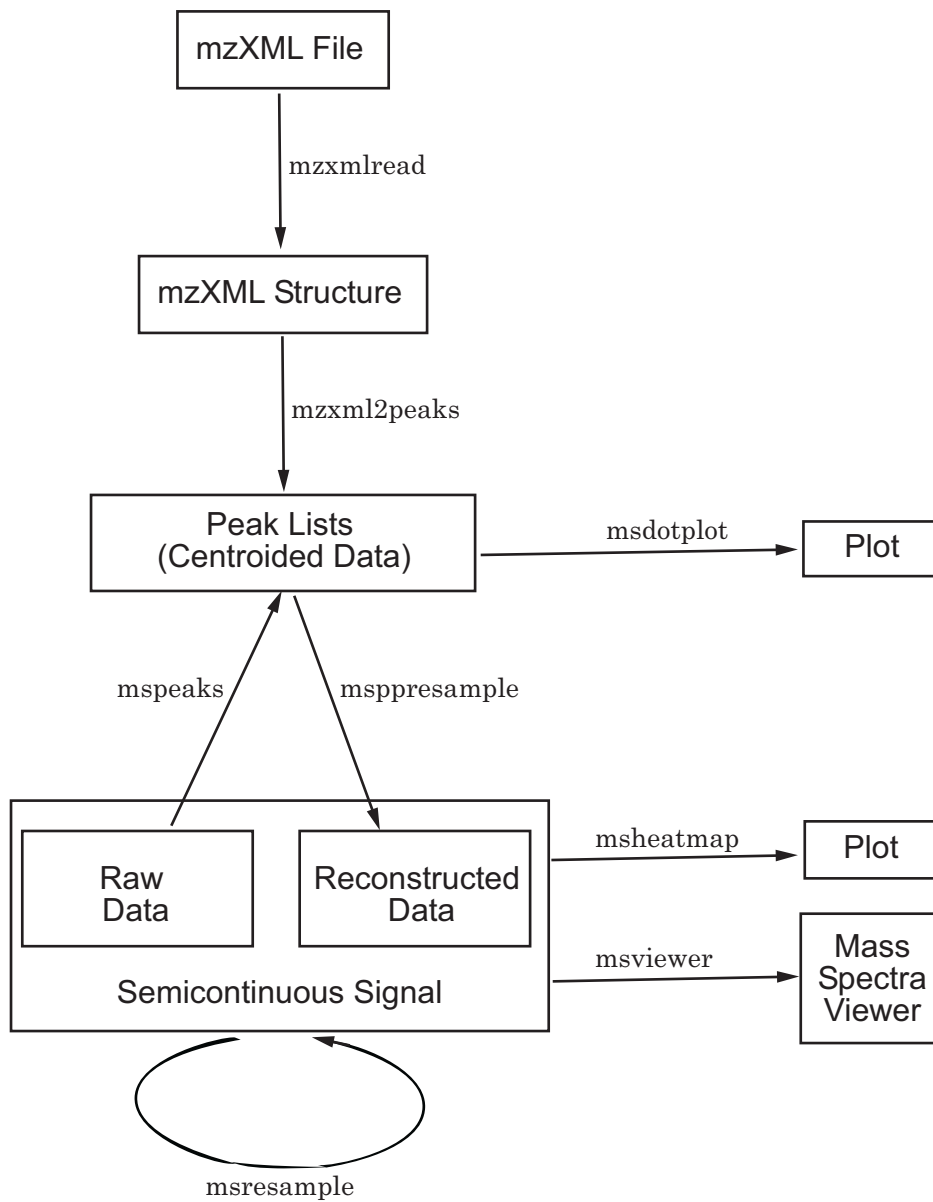
**Preprocessing raw data** — Resample high-resolution data to a lower resolution (`msresample`) where the extra data points are not needed. Correct the baseline (`msbackadj`). Align a spectrum to a set of reference masses



(`msalign`) and visually verify the alignment (`msheatmap`). Normalize the area between spectra for comparing (`msnorm`), and filter out noise (`mslowess` and `mssgolay`).

**Spectrum analysis** — Load spectra into a GUI (`msviewer`) for selecting mass peaks and further analysis.

The following graphic illustrates the roles of the various mass spectrometry functions in the toolbox.



## Graph Theory Functions

Graph theory functions in the toolbox apply basic graph theory algorithms to sparse matrices. A sparse matrix represents a graph, any nonzero entries in the matrix represent the edges of the graph, and the values of these entries represent the associated weight (cost, distance, length, or capacity) of the edge. Graph algorithms that use the weight information will cancel the edge if a NaN or an Inf is found. Graph algorithms that do not use the weight information will consider the edge if a NaN or an Inf is found, because these algorithms look only at the connectivity described by the sparse matrix and not at the values stored in the sparse matrix.

Sparse matrices can represent four types of graphs:

- **Directed Graph** — Sparse matrix, either double real or logical. Row (column) index indicates the source (target) of the edge. Self-loops (values in the diagonal) are allowed, although most of the algorithms ignore these values.
- **Undirected Graph** — Lower triangle of a sparse matrix, either double real or logical. An algorithm expecting an undirected graph ignores values stored in the upper triangle of the sparse matrix and values in the diagonal.
- **Direct Acyclic Graph (DAG)** — Sparse matrix, double real or logical, with zero values in the diagonal. While a zero-valued diagonal is a requirement of a DAG, it does not guarantee a DAG. An algorithm expecting a DAG will *not* test for cycles because this will add unwanted complexity.
- **Spanning Tree** — Undirected graph with no cycles and with one connected component.

There are no attributes attached to the graphs; sparse matrices representing all four types of graphs can be passed to any graph algorithm. All functions will return an error on nonsquare sparse matrices.

Graph algorithms do not pretest for graph properties because such tests can introduce a time penalty. For example, there is an efficient shortest path algorithm for DAG, however testing if a graph is acyclic is expensive compared to the algorithm. Therefore, it is important to select a graph theory function and properties appropriate for the type of the graph represented by your input matrix. If the algorithm receives a graph type that differs from what it expects, it will either:

- Return an error when it reaches an inconsistency. For example, if you pass a cyclic graph to the `graphshortestpath` function and specify `Acyclic` as the method property.
- Produce an invalid result. For example, if you pass a directed graph to a function with an algorithm that expects an undirected graph, it will ignore values in the upper triangle of the sparse matrix.

The graph theory functions include `graphallshortestpaths`, `graphconncomp`, `graphisdag`, `graphisomorphism`, `graphissspantree`, `graphmaxflow`, `graphminspantree`, `graphpred2path`, `graphshortestpath`, `graphtopoorder`, and `graphtraverse`.

## Graph Visualization

The toolbox includes functions, objects, and methods for creating, viewing, and manipulating graphs such as interactive maps, hierarchy plots, and pathways. This allows you to view relationships between data.

The object constructor function (`biograph`) lets you create a biograph object to hold graph data. Methods of the biograph object let you calculate the position of nodes (`dolayout`), draw the graph (`view`), get handles to the nodes and edges (`getnodesbyid` and `getedgesbynodeid`) to further query information, and find relations between the nodes (`getancestors`, `getdescendants`, and `getrelatives`). There are also methods that apply basic graph theory algorithms to the biograph object.

Various properties of a biograph object let you programmatically change the properties of the rendered graph. You can customize the node representation, for example, drawing pie charts inside every node (`CustomNodeDrawFcn`). Or you can associate your own callback functions to nodes and edges of the graph, for example, opening a Web page with more information about the nodes (`NodeCallback` and `EdgeCallback`).

## Statistical Learning and Visualization

You can classify and identify features in data sets, set up cross-validation experiments, and compare different classification methods.

The toolbox provides functions that build on the classification and statistical learning tools in the Statistics Toolbox software (`classify`, `kmeans`, and `treefit`).

These functions include imputation tools (`knnimpute`), and K-nearest neighbor classifiers (`knnclassify`).

Other functions include set up of cross-validation experiments (`crossvalind`) and comparison of the performance of different classification methods (`classperf`). In addition, there are tools for selecting diversity and discriminating features (`rankfeatures`, `randfeatures`).

## Prototyping and Development Environment

The MATLAB environment lets you prototype and develop algorithms and easily compare alternatives.

- **Integrated environment** — Explore biological data in an environment that integrates programming and visualization. Create reports and plots with the built-in functions for mathematics, graphics, and statistics.
- **Open environment** — Access the source code for the toolbox functions. The toolbox includes many of the basic bioinformatics functions you will need to use, and it includes prototypes for some of the more advanced functions. Modify these functions to create your own custom solutions.
- **Interactive programming language** — Test your ideas by typing functions that are interpreted interactively with a language whose basic data element is an array. The arrays do not require dimensioning and allow you to solve many technical computing problems,

Using matrices for sequences or groups of sequences allows you to work efficiently and not worry about writing loops or other programming controls.

- **Programming tools** — Use a visual debugger for algorithm development and refinement and an algorithm performance profiler to accelerate development.

## Data Visualization

You can visually compare pairwise sequence alignments, multiply aligned sequences, gene expression data from microarrays, and plot nucleic acid and

protein characteristics. The 2-D and volume visualization features let you create custom graphical representations of multidimensional data sets. You can also create montages and overlays, and export finished graphics to an Adobe® PostScript® image file or copy directly into Microsoft® PowerPoint®.

## **Algorithm Sharing and Application Deployment**

The open MATLAB environment lets you share your analysis solutions with other users, and it includes tools to create custom software applications. With the addition of MATLAB Compiler software, you can create standalone applications independent of the MATLAB environment, and, with the addition of MATLAB Builder NE software, you can create GUIs and standalone applications within other programming environments.

- **Share algorithms with other users** — You can share data analysis algorithms created in the MATLAB language across all supported platforms by giving files to other users. You can also create GUIs within the MATLAB environment using the Graphical User Interface Development Environment (GUIDE).
- **Deploy MATLAB GUIs** — Create a GUI within the MATLAB environment using GUIDE, and then use MATLAB Compiler software to create a standalone GUI application that runs separately from the MATLAB environment.
- **Create dynamic link libraries (DLLs)** — Use MATLAB Compiler software to create DLLs for your functions, and then link these libraries to other programming environments such as C and C++.
- **Create COM objects** — Use MATLAB Builder NE software to create COM objects, and then use a COM-compatible programming environment (Visual Basic®) to create a standalone application.
- **Create Excel add-ins** — Use MATLAB Builder EX software to create Excel add-in functions, and then use these functions with Excel spreadsheets.
- **Create Java classes** — Use MATLAB Builder JA software to automatically generate Java classes from algorithms written in the MATLAB programming language. You can run these classes outside the MATLAB environment.

## Importing and Exploring Bioinformatic Data from Microsoft Excel

If you have bioinformatic data in a Microsoft Excel spreadsheet, you can use Spreadsheet Link EX software to connect Excel with the MATLAB Workspace to exchange data and to use MATLAB and Bioinformatics Toolbox computational and visualization functions.

---

**Note** The following example assumes you have Spreadsheet Link EX software installed on your system.

---

The file used in the following example contains data from DeRisi, J.L., Iyer, V.R., and Brown, P.O. (Oct. 24, 1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278(5338), 680–686. PMID: 9381177. The data has been filtered using the steps described in the Gene Expression Profile Analysis demo.

- 1** If you have not already done so, modify your system path to include the MATLAB root directory as described in “Modifying Your System Path” in the Spreadsheet Link EX documentation.
- 2** If you have not already done so, enable the Spreadsheet Link EX Add-In as described in “Configuring the Spreadsheet Link EX Software” in the Spreadsheet Link EX documentation.
- 3** Close MATLAB if you have it open.
- 4** Start Microsoft Excel. MATLAB and Spreadsheet Link EX software open also.
- 5** From Excel, open the following file provided with the Bioinformatics Toolbox software:

```
matlabroot\toolbox\bioinfo\biodemos\Filtered_Yeastdata.xls
```

---

**Note** The notation *matlabroot* is the MATLAB root directory, which is the directory where the MATLAB software is installed on your system.

---


---

**Note** If the Security Warning appears, click **Enable Macros**.

---

- 6** After opening the provided file, if you receive a Microsoft® Visual Basic® error, you may need to do one or both of the following:
- In the Excel software, enable macros. If you are running Excel 2003 or earlier software, select **Tools > Macro > Security**. If you are running Excel 2007 or 2010 software, select the **Developer** tab, then select **Macro Security** from the Code group. (If the **Developer** tab is not displayed on the Excel ribbon, consult Excel Help to display it.)
  - Enable the Spreadsheet Link EX software as a Reference in the Microsoft Visual Basic Editor, making sure to select the path you selected in step 2.
- 7** Note that cells J5, J6, J7, and J12 of the spreadsheet contain formulas using Spreadsheet Link EX functions `MLPutMatrix` and `MLEvalString`.

---

**Tip** To view a cell's formula, select the cell, then view the formula in the formula bar  \_\_\_\_\_ at the top of the Excel window.

---

Cells J5, J6, and J7 each create a MATLAB variable from the data in the spreadsheet, and cell J12 runs the Bioinformatics Toolbox `clustergram` function using these three variables as input. For more information on adding formulas using Spreadsheet Link EX functions, see “Entering Functions into Worksheet Cells” in the Spreadsheet Link EX documentation.



Cells J5, J6, and J7 contain formulas that use the MLPutMatrix function to create three MATLAB variables.


Cell J12 contains a formula that uses the MLEvalString function to run the Bioinformatics Toolbox function clustergram.

Push the data into 3 MATLAB variables			
	0 <== MLPutMatrix("data",B4:H617)		
	0 <== MLPutMatrix("Genes",A4:A617)		
	0 <== MLPutMatrix("TimeSteps",B3:H3)		
Run the clustergram command on the data using the 3 variables			
	0 <== MLEvalString("clustergram(data,'RowLabels',Genes,'ColumnLa		
Run the macro function Clustergram on the data using cell ranges			
	0 <== Clustergram(B4:H617,A4:A617,B3:H3)		

Cell J17 contains a formula that uses a macro function, Clustergram, created in Visual Basic Editor.

**8** Note that cell J17 contains a formula using a macro function **Clustergram**, which was created in the Visual Basic Editor. Running this macro does the same as the formulas in cells J5, J6, J7, and J12. Optionally, view the **Clustergram** macro function:

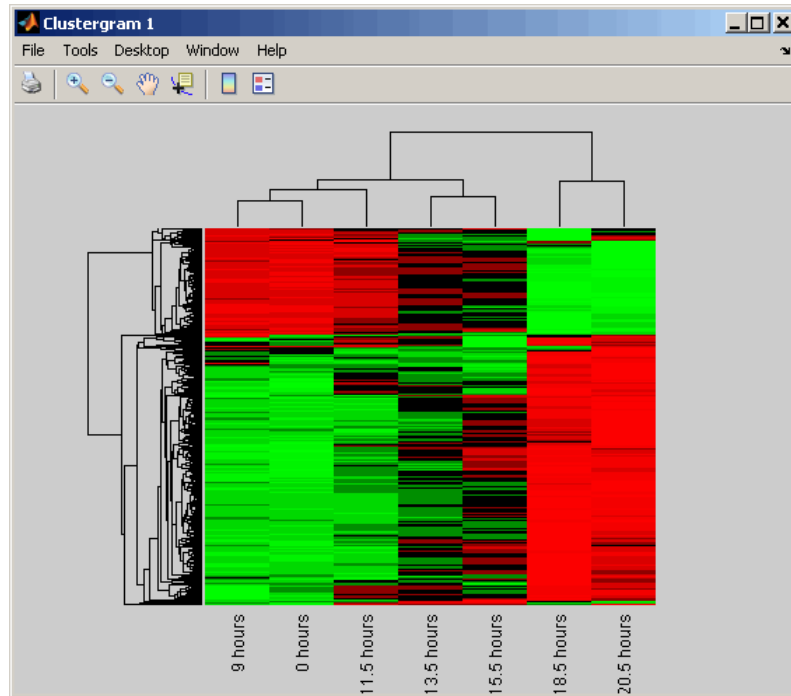
- If you are running Excel 2003 or earlier software, select **Tools > Macro > Visual Basic Editor**

- If you are running Excel 2007 or 2010 software, select the **Developer** tab, then select the Visual Basic button . (If the **Developer** tab is not displayed on the Excel ribbon, consult Excel Help to display it.)

For more information on creating macros using Visual Basic Editor, see “Examples: Using Spreadsheet Link EX Functions in Macros” in the Spreadsheet Link EX documentation.

- 9 Run the formula in cell J17 to analyze and visualize the data:
  - a Select cell **J17**.
  - b Press **F2**.
  - c Press **Enter**.

The macro function `Clustergram` runs creating three MATLAB variables (`data`, `Genes`, and `TimeSteps`) and displaying a Clustergram window containing dendrograms and a heat map of the data.



**10** Edit the formulas in cells J5 and J6 to analyze a subset of the data. Do this by editing the formulas' cell ranges to include data for only the first 30 genes:

- a** Select cell J5, then press F2 to display the formula for editing. Change H617 to H33, then press Enter.

```
=MLPutMatrix("data",B4:H33)
```

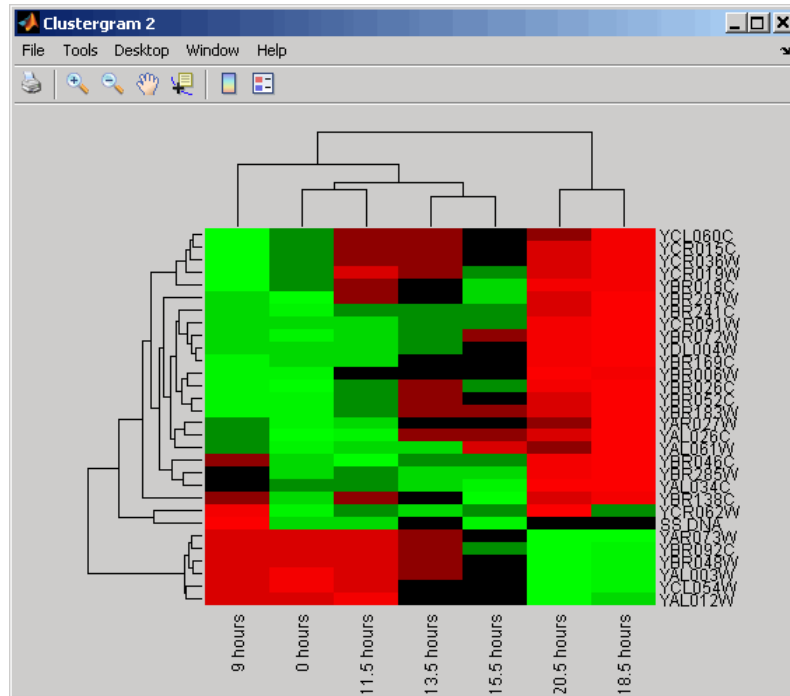
- b** Select cell J6, then press F2 to display the formula for editing. Change A617 to A33, then press Enter.

```
=MLPutMatrix("Genes",A4:A33)
```

**11** Run the formulas in cells J5, J6, J7, and J12 to analyze and visualize a subset of the data.

- a** Select cell J5, press F2, then press Enter.

- b** Select cell **J6**, press **F2**, then press **Enter**.
- c** Select cell **J7**, press **F2**, then press **Enter**.
- d** Select cell **J12**, press **F2**, then press **Enter**.



- 12** If you are running Excel 2003 or earlier software, use the commands in the Spreadsheet Link EX toolbar to interact with the data:
- a** Click-drag to select cells **B5** through **H7**, click **putmatrix** in the toolbar, type **YAGenes** for the variable name, then click **OK**. The variable **YAGenes** is added to the MATLAB Workspace as a 3-by-7 matrix.

0.305	0.146	-0.129	-0.444	-0.707	-1.499	-1.935
0.157	0.175	0.467	-0.379	-0.52	-1.279	-2.125
0.246	0.796	0.384	0.981	1.02	1.646	1.157

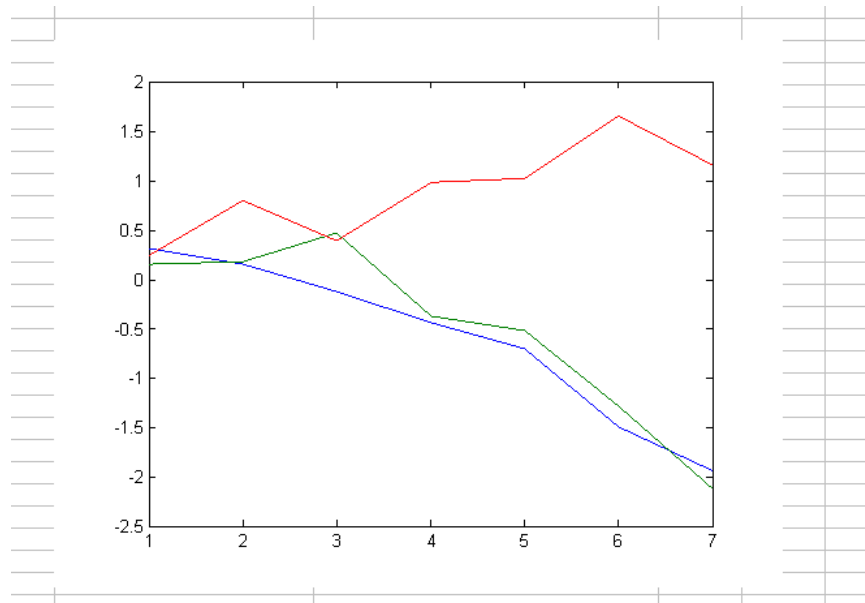
- b** Click **evalstring** in the toolbar, type **plot(YAGenes )** for the command, then click **OK**. A Figure window displays a plot of the data.

---

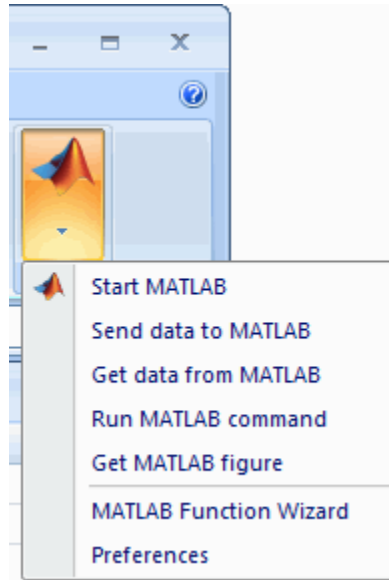
**Note** Make sure you use the ' (transpose) symbol when plotting the data in this step. You need to transpose the data in YAGenes so that it plots as three genes over seven time intervals.

---

- c** Select cell **J20**, then click **getfigure** in the toolbar. The figure is added to the spreadsheet



- 13** If you are running Excel 2007 or 2010 software, use the MATLAB group on the right side of the **Home** tab to interact with the data:



- a Click-drag to select cells **B5** through **H7**, then from the MATLAB group, select **Send data to MATLAB**, type **YAGenes** for the variable name, then click **OK**. The variable **YAGenes** is added to the MATLAB Workspace as a 3-by-7 matrix.

0.305	0.146	-0.129	-0.444	-0.707	-1.499	-1.935
0.157	0.175	0.467	-0.379	-0.52	-1.279	-2.125
0.246	0.796	0.384	0.981	1.02	1.646	1.157

- b From the MATLAB group, select **Run MATLAB command**, type **plot(YAGenes')** for the command, then click **OK**. A Figure window displays a plot of the data.

---

**Note** Make sure you use the ' (transpose) symbol when plotting the data in this step. You need to transpose the data in YAGenes so that it plots as three genes over seven time intervals.

---

- c Select cell **J20**, then from the MATLAB group, select **Get MATLAB figure**. The figure is added to the spreadsheet

## Creating get Functions

### In this section...

“What Are get Functions?” on page 1-29

“Creating the getpubmed Function” on page 1-30

## What Are get Functions?

Bioinformatics Toolbox includes several get functions that retrieve information from various Web databases. Additionally, with some basic MATLAB programming skills, you can create your own get function to retrieve information from a specific Web database.

The following procedure illustrates how to create a function to retrieve information from the NCBI PubMed database and read the information into a MATLAB structure. The NCBI PubMed database contains biomedical literature citations and abstracts.

NCBI PubMed A service of the U.S. National Library of Medicine and the National Institutes of Health  
www.pubmed.gov

All Databases PubMed Nucleotide Protein Genome Structure OMIM PMC Journals Books

Search PubMed for Go Clear [Advanced Search \(beta\)](#)

Limits Preview/Index History Clipboard Details

About Entrez  
Text Version

Entrez PubMed  
Overview  
Help | FAQ  
Tutorials  
New/Noteworthy  
E-Utilities

PubMed Services  
Journals Database  
MeSH Database  
Single Citation  
Matcher  
Batch Citation Matcher  
Clinical Queries  
Special Queries  
LinkOut  
My NCBI

**To get started with PubMed, enter one or more search terms.**

Search terms may be **topics, authors or journals.**

**My NCBI** Set up an automated PubMed update in fewer than five minutes.

1. Create a [My NCBI account](#).
2. Save your search.
3. Your PubMed updates can be e-mailed directly to you.

Read the [My NCBI Help](#) material to explore other options, such as automated updates of other databases, setting search filters, and highlighting search terms.

PubMed is a service of the [U.S. National Library of Medicine](#) that includes over 17 million citations from MEDLINE and other life science journals for biomedical articles back to the 1950s. PubMed includes links to full text articles and other related resources.

## Creating the getpubmed Function

The following procedure shows you how to create a function named `getpubmed` using the MATLAB Editor. This function will retrieve citation and abstract information from PubMed literature searches and write the data to a MATLAB structure.

Specifically, this function will take one or more search terms, submit them to the PubMed database for a search, then return a MATLAB structure or structure array, with each structure containing information for an article found by the search. The returned information will include a PubMed identifier, publication date, title, abstract, authors, and citation.

The function will also include property name/property value pairs that let the user of the function limit the search by publication date and limit the number of records returned.

**1** From MATLAB, open the MATLAB Editor by selecting **File > New > Function**.

**2** Define the `getpubmed` function, its input arguments, and return values by typing:

```
function pmstruct = getpubmed(searchterm,varargin)
% GETPUBMED Search PubMed database & write results to MATLAB structure
```

**3** Add code to do some basic error checking for the required input `SEARCHTERM`.

```
% Error checking for required input SEARCHTERM
if(nargin<1)
    error('GETPUBMED:NotEnoughInputArguments',...
        'SEARCHTERM is missing.');
```

**4** Create variables for the two property name/property value pairs, and set their default values.

```
% Set default settings for property name/value pairs,
% 'NUMBEROFRECORDS' and 'DATEOFPUBLICATION'
maxnum = 50; % NUMBEROFRECORDS default is 50
pubdate = ''; % DATEOFPUBLICATION default is an empty string
```



- 5** Add code to parse the two property name/property value pairs if provided as input.

```
% Parsing the property name/value pairs
num_argin = numel(varargin);
for n = 1:2:num_argin
    arg = varargin{n};
    switch lower(arg)

        % If NUMBEROFRECORDS is passed, set MAXNUM
        case 'numberofrecords'
            maxnum = varargin{n+1};

        % If DATEOFPUBLICATION is passed, set PUBDATE
        case 'dateofpublication'
            pubdate = varargin{n+1};

    end
end
```

- 6** You access the PubMed database through a search URL, which submits a search term and options, and then returns the search results in a specified format. This search URL is comprised of a base URL and defined parameters. Create a variable containing the base URL of the PubMed database on the NCBI Web site.

```
% Create base URL for PubMed db site
baseSearchURL = 'http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=search';
```

- 7** Create variables to contain five defined parameters that the `getpubmed` function will use, namely, `db` (database), `term` (search term), `report` (report type, such as MEDLINE<sup>®</sup>), `format` (format type, such as text), and `dispmax` (maximum number of records to display).

```
% Set db parameter to pubmed
dbOpt = '&db=pubmed';

% Set term parameter to SEARCHTERM and PUBDATE
% (Default PUBDATE is '')
termOpt = ['&term=', searchterm, '+AND+', pubdate];
```

```
% Set report parameter to medline
reportOpt = '&report=medline';

% Set format parameter to text
formatOpt = '&format=text';

% Set dispmax to MAXNUM
% (Default MAXNUM is 50)
maxOpt = ['&dispmax=', num2str(maxnum)];
```

- 8** Create a variable containing the search URL from the variables created in the previous steps.

```
% Create search URL
searchURL = [baseSearchURL, dbOpt, termOpt, reportOpt, formatOpt, maxOpt];
```

- 9** Use the `urlread` function to submit the search URL, retrieve the search results, and return the results (as text in the MEDLINE report type) in `medlineText`, a character array.

```
medlineText = urlread(searchURL);
```

- 10** Use the MATLAB `regexp` function and regular expressions to parse and extract the information in `medlineText` into `hits`, a cell array, where each cell contains the MEDLINE-formatted text for one article. The first input is the character array to search, the second input is a search expression, which tells the `regexp` function to find all records that start with PMID-, while the third input, 'match', tells the `regexp` function to return the actual records, rather than the positions of the records.

```
hits = regexp(medlineText, 'PMID-.*(?=PMID|</pre>$)', 'match');
```

- 11** Instantiate the `pmstruct` structure returned by `getpubmed` to contain six fields.

```
pmstruct = struct('PubMedID', '', 'PublicationDate', '', 'Title', '', ...
                  'Abstract', '', 'Authors', '', 'Citation', '');
```

- 12** Use the MATLAB `regexp` function and regular expressions to loop through each article in `hits` and extract the PubMed ID, publication date, title,

abstract, authors, and citation. Place this information in the `pmstruct` structure array.

```
for n = 1:numel(hits)
    pmstruct(n).PubMedID = regexp(hits{n}, '(?<=PMID- ).*?(?=\n)', 'match', 'once');
    pmstruct(n).PublicationDate = regexp(hits{n}, '(?<=DP - ).*?(?=\n)', 'match', 'once');
    pmstruct(n).Title = regexp(hits{n}, '(?<=TI - ).*?(?=PG -|AB -)', 'match', 'once');
    pmstruct(n).Abstract = regexp(hits{n}, '(?<=AB - ).*?(?=AD -)', 'match', 'once');
    pmstruct(n).Authors = regexp(hits{n}, '(?<=AU - ).*?(?=\n)', 'match');
    pmstruct(n).Citation = regexp(hits{n}, '(?<=SO - ).*?(?=\n)', 'match', 'once');
end
```

### 13 Select **File > Save As**.

When you are done, your file should look similar to the `getpubmed.m` file included with the Bioinformatics Toolbox software. The sample `getpubmed.m` file, including help, is located at:

```
matlabroot\toolbox\bioinfo\biodeemos\getpubmed.m
```

---

**Note** The notation *matlabroot* is the MATLAB root directory, which is the directory where the MATLAB software is installed on your system.

---

## Extracting Information from Large Multi-Entry Text Files

### In this section...

“Overview” on page 1-34

“What Files Can You Access?” on page 1-34

“Before You Begin” on page 1-35

“Creating a BioIndexedFile Object to Access Your Source File” on page 1-36

“Determining the Number of Entries Indexed By a BioIndexedFile Object” on page 1-37

“Retrieving Entries from Your Source File” on page 1-37

“Reading Entries from Your Source File” on page 1-38

### Overview

Many biological experiments produce huge data files that are difficult to access due to their size, which can cause memory issues when reading the file into the MATLAB Workspace. You can construct a `BioIndexedFile` object to access the contents of a large text file (up to 4 GB) containing nonuniform size entries, such as sequences, annotations, and cross-references to data sets. The `BioIndexedFile` object lets you quickly and efficiently access this data without loading the source file into memory.

You can use the `BioIndexedFile` object to access individual entries or a subset of entries when the source file is too big to fit into memory. You can access entries using indices or keys. You can read and parse one or more entries using provided interpreters or a custom interpreter function.

Use the `BioIndexedFile` object in conjunction with your large source file to:

- Access a subset of the entries for validation or further analysis.
- Parse entries using a custom interpreter function.

### What Files Can You Access?

You can use the `BioIndexedFile` object to access large text files up to 4 GB in size.

Your source file can have these application-specific formats:

- FASTA
- FASTQ
- SAM

Your source file can also have these general formats:

- **Table** — Tab-delimited table with multiple columns. Keys can be in any column. Rows with the same key are considered separate entries.
- **Multi-row Table** — Tab-delimited table with multiple columns. Keys can be in any column. Contiguous rows with the same key are considered a single entry. Noncontiguous rows with the same key are considered separate entries.
- **Flat** — Flat file with concatenated entries separated by a character string, typically `//`. Within an entry, the key is separated from the rest of the entry by a white space.

## Before You Begin

Before constructing a `BioIndexedFile` object, locate your source file on your hard drive or a local network.

When you construct a `BioIndexedFile` object from your source file for the first time, you also create an auxiliary index file, which by default is saved to the same location as your source file. However, if your source file is in a read-only location, you can specify a different location to save the index file.

---

**Tip** If you construct a `BioIndexedFile` object from your source file on subsequent occasions, it takes advantage of the existing index file, which saves time. However, the index file must be in the same location or a location specified by the subsequent construction syntax.

---

---

**Tip** If insufficient memory is not an issue when accessing your source file, you may want to try an appropriate read function, such as `genbankread`, for importing data from GenBank files. For a complete list of read functions in the Bioinformatics Toolbox, see “Data Formats and Databases” in the Bioinformatics Toolbox Reference.

Additionally, several read functions such as `fastaread`, `fastqread`, `samread`, and `sffread` include a `Blockread` property, which lets you read a subset of entries from a file, thus saving memory.

---

## Creating a `BioIndexedFile` Object to Access Your Source File

To construct a `BioIndexedFile` object from a multi-row table file:

- 1 Create a variable containing the full absolute path of your source file. For your source file, use the `yeastgenes.sgd` file, which is included with the Bioinformatics Toolbox software.

```
sourcefile = which('yeastgenes.sgd');
```

- 2 Use the `BioIndexedFile` constructor function to construct a `BioIndexedFile` object from the `yeastgenes.sgd` source file, which is a multi-row table file. Save the index file in the Current Folder. Indicate that the source file keys are in column 3. Also, indicate that the header lines in the source file are prefaced with `!`, so the constructor ignores them.

```
gene2goObj = BioIndexedFile('mrtab', sourcefile, '.', ...  
                           'KeyColumn', 3, 'HeaderPrefix', '!')
```

The `BioIndexedFile` constructor function constructs `gene2goObj`, a `BioIndexedFile` object, and also creates an index file with the same name as the source file, but with an `IDX` extension. It stores this index file in the Current Folder because we specified this location. However, the default location for the index file is the same location as the source file.

---

**Caution** Do not modify the index file. If you modify it, you can get invalid results. Also, the constructor function cannot use a modified index file to construct future objects from the associated source file.

---

## Determining the Number of Entries Indexed By a `BioIndexedFile` Object

To determine the number of entries indexed by a `BioIndexedFile` object, use the `NumEntries` property of the `BioIndexedFile` object. For example, for the `gene2goObj` object:

```
gene2goObj.NumEntries
```

```
ans =
```

```
6476
```

---

**Note** For a list and description of all properties of a `BioIndexedFile` object, see `BioIndexedFile` class.

---

## Retrieving Entries from Your Source File

Retrieve entries from your source file using either:

- The index of the entry
- The entry key

### Retrieving Entries Using Indices

Use the `getEntryByIndex` method to retrieve a subset of entries from your source file that correspond to specified indices. For example, retrieve the first 12 entries from the `yeastgenes.sgd` source file:

```
subset_entries = getEntryByIndex(gene2goObj, [1:12]);
```

## Retrieving Entries Using Keys

Use the `getEntryByKey` method to retrieve a subset of entries from your source file that are associated with specified keys. For example, retrieve all entries with keys of AAC1 and AAD10 from the `yeastgenes.sgd` source file:

```
subset_entries = getEntryByKey(gene2goObj, {'AAC1' 'AAD10'});
```

The output `subset_entries` is a single string of concatenated entries. Because the keys in the `yeastgenes.sgd` source file are not unique, this method returns all entries that have a key of AAC1 or AAD10.

## Reading Entries from Your Source File

The `BioIndexedFile` object includes a `read` method, which you can use to read and parse a subset of entries from your source file. The `read` method parses the entries using an interpreter function specified by the `Interpreter` property of the `BioIndexedFile` object.

## Setting the Interpreter Property

Before using the `read` method, make sure the `Interpreter` property of the `BioIndexedFile` object is set appropriately.

<b>If you constructed a <code>BioIndexedFile</code> object from ...</b>	<b>The <code>Interpreter</code> property ...</b>
A source file with an application-specific format (FASTA, FASTQ, or SAM)	By default is a handle to a function appropriate for that file type and typically does not require you to change it.
A source file with a table, multi-row table, or flat format	By default is <code>[]</code> , which means the interpreter is an anonymous function in which the output is equivalent to the input. You can change this to a handle to a function that accepts a single string of one or more concatenated entries and returns a structure or an array of structures containing the interpreted data.



There are two ways to set the `Interpreter` property of the `BioIndexedFile` object:

- When constructing the `BioIndexedFile` object, use the `Interpreter` property name/property value pair
- After constructing the `BioIndexedFile` object, set the `Interpreter` property

---

**Note** For more information on setting the `Interpreter` property of a `BioIndexedFile` object, see `BioIndexedFile` class.

---

## Reading a Subset of Entries

The `read` method reads and parses a subset of entries that you specify using either entry indices or keys.

### Example

To quickly find all the gene ontology (GO) terms associated with a particular gene because the entry keys are gene names:

- 1 Set the `Interpreter` property of the `gene2goObj` `BioIndexedFile` object to a handle to a function that reads entries and returns only the column containing the GO term. In this case the interpreter is a handle to an anonymous function that accepts strings and extracts strings that start with the characters `GO`.

```
gene2goObj.Interpreter = @(x) regexp(x, 'GO:\d+', 'match')
```

- 2 Read only the entries that have a key of `YAT2`, and return their GO terms.

```
GO_YAT2_entries = read(gene2goObj, 'YAT2')
```

```
GO_YAT2_entries =
```

```
'GO:0004092' 'GO:0005737' 'GO:0006066' 'GO:0006066' 'GO:0009437'
```



# Sequence Analysis

---

Sequence analysis is the process you use to find information about a nucleotide or amino acid sequence using computational methods. Common tasks in sequence analysis are identifying genes, determining the similarity of two genes, determining the protein coded by a gene, and determining the function of a gene by finding a similar gene in another organism with a known function.

- “Example: Sequence Statistics” on page 2-2
- “Example: Sequence Alignment” on page 2-22
- “Sequence Tool” on page 2-42
- “Multiple Sequence Alignment Viewer” on page 2-56
- “Storing and Managing Short-Read Sequence Data in Objects” on page 2-63

## Example: Sequence Statistics

In this section...
“Overview of Example” on page 2-2
“Determining Nucleotide Content” on page 2-2
“Reading Sequence Information” on page 2-5
“Determining Nucleotide Composition” on page 2-6
“Determining Codon Composition” on page 2-11
“Open Reading Frames” on page 2-15
“Amino Acid Conversion and Composition” on page 2-18

### Overview of Example

After sequencing a piece of DNA, one of the first tasks is to investigate the nucleotide content in the sequence. Starting with a DNA sequence, this example uses sequence statistics functions to determine mono-, di-, and trinucleotide content, and to locate open reading frames.

### Determining Nucleotide Content

The following procedure illustrates how to use the MATLAB Help browser to search the Web for information. In this example you are interested in studying the human mitochondrial genome. While many genes that code for mitochondrial proteins are found in the cell nucleus, the mitochondrial has genes that code for proteins used to produce energy.

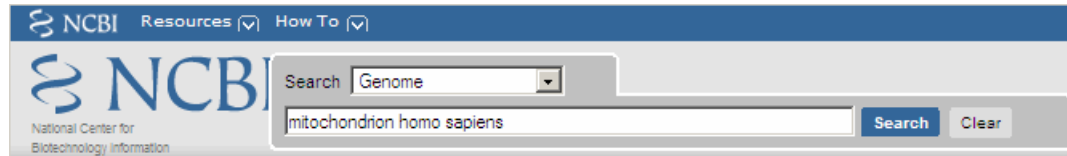
First research information about the human mitochondria and find the nucleotide sequence for the genome. Next, look at the nucleotide content for the entire sequence. And finally, determine open reading frames and extract specific gene sequences.

- 1 Use the MATLAB Help browser to explore the Web. In the MATLAB Command Window, type

```
web('http://www.ncbi.nlm.nih.gov/')
```

A separate browser window opens with the home page for the NCBI Web site.

- 2 Search the NCBI Web site for information. For example, to search for the human mitochondrion genome, from the **Search** list, select **Genome**, and in the **Search** list, enter mitochondrion homo sapiens.



The NCBI Web search returns a list of links to relevant pages.

 A screenshot of the NCBI search results page. The top navigation bar includes 'PubMed', 'Nucleotide', 'Protein', 'Genome', 'Structure', 'OMIM', and 'PMC'. The search bar shows 'Genome' selected and 'mitochondrion homo sapiens' entered. Below the search bar are buttons for 'Limits', 'Preview/Index', 'History', 'Clipboard', and 'Details'. The display options are set to 'Summary', 'Show 20', and 'Send to'. The results section shows 'All: 49' and 'Items 1 - 20 of 49'. The first result is '1: [NC\\_003415](#) Ancylostoma duodenale mitochondrion, complete genome'. Below the result title are details: 'DNA; circular; Length: 13,721 nt', 'Organelle: mitochondrion', and 'Created: 2002/02/21'. A 'Links' button is visible to the right of the result.

- 3 Select a result page. For example, click the link labeled **NC\_012920**.

The MATLAB Help browser displays the NCBI page for the human mitochondrial genome.

NCBI **Genome** My NCBI [Sign In] [Register]

PubMed Nucleotide Protein Genome Structure OMIM PMC Journals Books

Search Genome for [ ] Go Clear

Limits Preview/Index History Clipboard Details

Display Overview Show 20 Send to

All: 1

**Genome** > *Eukaryota* > *Homo sapiens mitochondrion, complete genome* Links

Lineage: [Eukaryota](#); [Fungi/Metazoa group](#); [Metazoa](#); [Eumetazoa](#); [Bilateria](#); [Coelomata](#); [Deuterostomia](#); [Chordata](#); [Craniata](#); [Vertebrata](#); [Gnathostomata](#); [Teleostomi](#); [Euteleostomi](#); [Sarcopterygii](#); [Tetrapoda](#); [Amniota](#); [Mammalia](#); [Theria](#); [Eutheria](#); [Euarchontoglires](#); [Primates](#); [Haplorrhini](#); [Simiiformes](#); [Catarrhini](#); [Hominoidea](#); [Hominidae](#); [Homininae](#); [Homo](#); [Homo sapiens](#)

Genome Info:	Features:	BLAST homologs:	Links:	Review Info:
Refseq: <a href="#">NC_012920</a>	Genes: <a href="#">37</a>	COG	<a href="#">Genome Project</a>	Publications: <a href="#">[2]</a>
GenBank: <a href="#">J01415</a>	Protein coding: <a href="#">13</a>	TaxMap	Refseq FTP	Refseq Status: PROVISIONAL
Length: <b>16,569 nt</b>	Structural RNAs: <a href="#">24</a>	<a href="#">TaxPlot</a>	GenBank FTP	Seq. Status: <b>Completed</b>
GC Content: <b>44%</b>	Pseudo genes: <b>None</b>	<a href="#">GenePlot</a>	BLAST	Sequencing center: <a href="#">Center for Molecular and Mitochondrial Medicine and Genetics (MAMMAG) University of California, University of California, Irvine, Mitomap.org, USA, Irvine</a>
% Coding: <b>68%</b>	Others: <b>30</b>	gMap	TraceAssembly	Completed: <b>2009/07/08</b>
Topology: <b>circular</b>	Contigs: <b>None</b>		CDD	<a href="#">Organism Group</a>
Molecule: <b>dsDNA</b>			Other genomes for species: <a href="#">5683</a>	

Gene Classification based on [COG functional categories](#)

Search gene, GeneID or locus\_tag:



Click [here](#) for Sequence Viewer presentation (base sequence and aligned amino acids) of selected region

Display Overview Show 20 Send to

## Reading Sequence Information

The following procedure illustrates how to find a nucleotide sequence in a public database and read the sequence information into the MATLAB environment. Many public databases for nucleotide sequences are accessible from the Web. The MATLAB Command Window provides an integrated environment for bringing sequence information into the MATLAB environment.

The consensus sequence for the human mitochondrial genome has the GenBank accession number NC\_012920. Since the whole GenBank entry is quite large and you might only be interested in the sequence, you can get just the sequence information.

- 1 Get sequence information from a Web database. For example, to retrieve sequence information for the human mitochondrial genome, in the MATLAB Command Window, type

```
mitochondria = getgenbank('NC_012920','SequenceOnly',true)
```

The `getgenbank` function retrieves the nucleotide sequence from the GenBank database and creates a character array.

```
mitochondria =
GATCACAGGTCTATCACCCCTATTAACCACTCACGGGAGCTCTCCATGCAT
TTGGTATTTTCGTCTGGGGGGTGTGCACGCGATAGCATTGCGAGACGCTG
GAGCCGGAGCACCCCTATGTGCGAGTATCTGTCTTTGATTTCCTGCCTCATT
CTATTATTTATCGCACCTACGTTCAATATTACAGGCGAACATACCTACTA
AAGT . . .
```

- 2 If you don't have a Web connection, you can load the data from a MAT file included with the Bioinformatics Toolbox software, using the command

```
load mitochondria
```

The `load` function loads the sequence `mitochondria` into the MATLAB Workspace.

- 3 Get information about the sequence. Type

```
whos mitochondria
```

Information about the size of the sequence displays in the MATLAB Command Window.

Name	Size	Bytes	Class	Attributes
mitochondria	1x16569	33138	char	

## Determining Nucleotide Composition

The following procedure illustrates how to determine the monomers and dimers, and then visualize data in graphs and bar plots. Sections of a DNA sequence with a high percent of A+T nucleotides usually indicate intergenic parts of the sequence, while low A+T and higher G+C nucleotide percentages indicate possible genes. Many times high CG dinucleotide content is located before a gene.

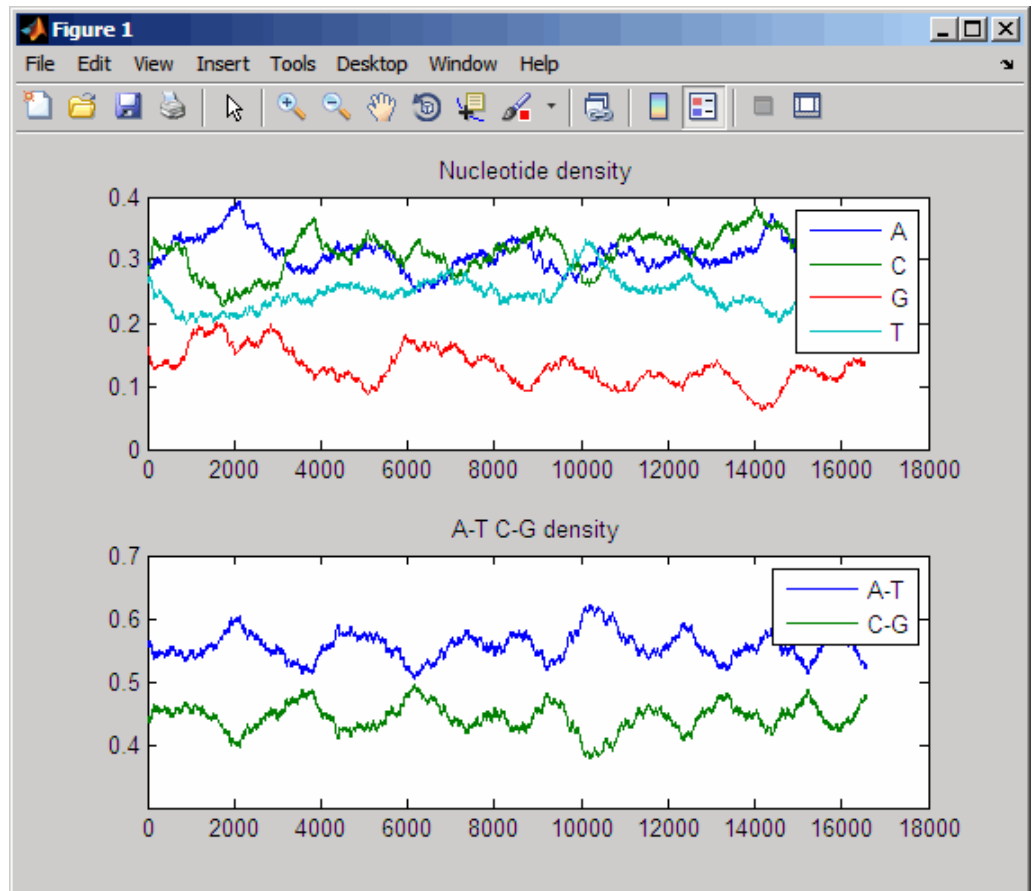
After you read a sequence into the MATLAB environment, you can use the sequence statistics functions to determine if your sequence has the characteristics of a protein-coding region. This procedure uses the human mitochondrial genome as an example. See “Reading Sequence Information” on page 2-5.

- 1 Plot monomer densities and combined monomer densities in a graph. In the MATLAB Command Window, type

```
ntdensity(mitochondria)
```

This graph shows that the genome is A+T rich.





**2** Count the nucleotides using the `basecount` function.

```
basecount(mitochondria)
```

A list of nucleotide counts is shown for the 5'-3' strand.

```
ans =
  A: 5124
  C: 5181
  G: 2169
  T: 4094
```

- 3** Count the nucleotides in the reverse complement of a sequence using the `seqrcomplement` function.

```
basecount(seqrcomplement(mitochondria))
```

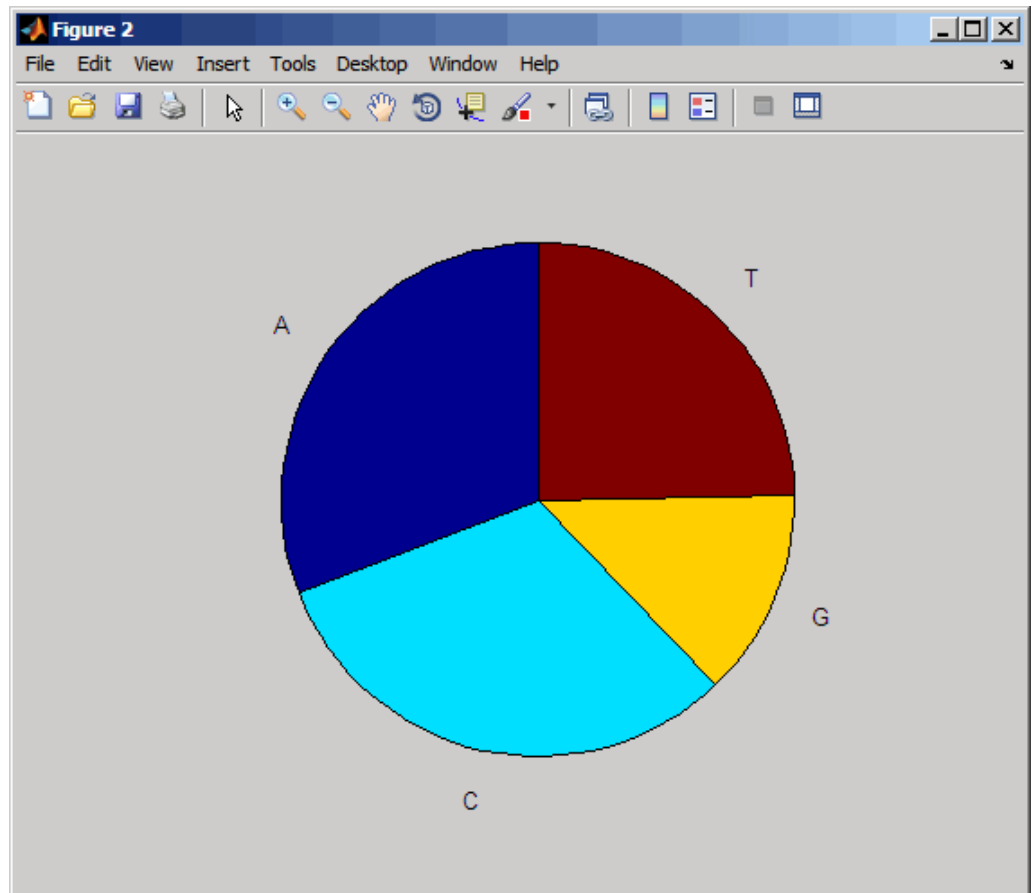
As expected, the nucleotide counts on the reverse complement strand are complementary to the 5'-3' strand.

```
ans =  
  A: 4094  
  C: 2169  
  G: 5181  
  T: 5124
```

- 4** Use the function `basecount` with the `chart` option to visualize the nucleotide distribution.

```
figure  
basecount(mitochondria, 'chart', 'pie');
```

A pie chart displays in the MATLAB Figure window.



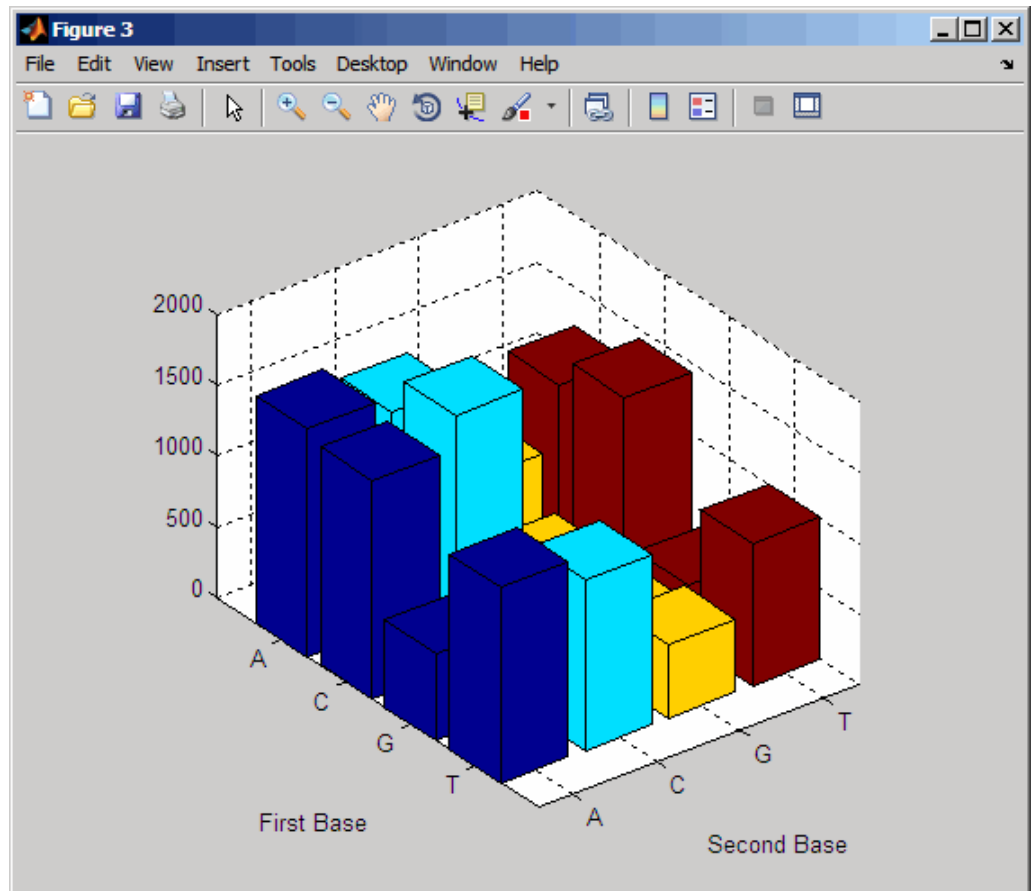
- 5 Count the dimers in a sequence and display the information in a bar chart.

```
figure  
dimercount(mitochondria,'chart','bar')
```

```
ans =
```

```
AA: 1604  
AC: 1495  
AG: 795  
AT: 1230
```

CA: 1534  
CC: 1771  
CG: 435  
CT: 1440  
GA: 613  
GC: 711  
GG: 425  
GT: 419  
TA: 1373  
TC: 1204  
TG: 513  
TT: 1004



## Determining Codon Composition

The following procedure illustrates how to look at codons for the six reading frames. Trinucleotides (codon) code for an amino acid, and there are 64 possible codons in a nucleotide sequence. Knowing the percent of codons in your sequence can be helpful when you are comparing with tables for expected codon usage.

After you read a sequence into the MATLAB environment, you can analyze the sequence for codon composition. This procedure uses the human mitochondria genome as an example. See “Reading Sequence Information” on page 2-5.

- 1 Count codons in a nucleotide sequence. In the MATLAB Command Window, type

```
codoncount(mitochondria)
```

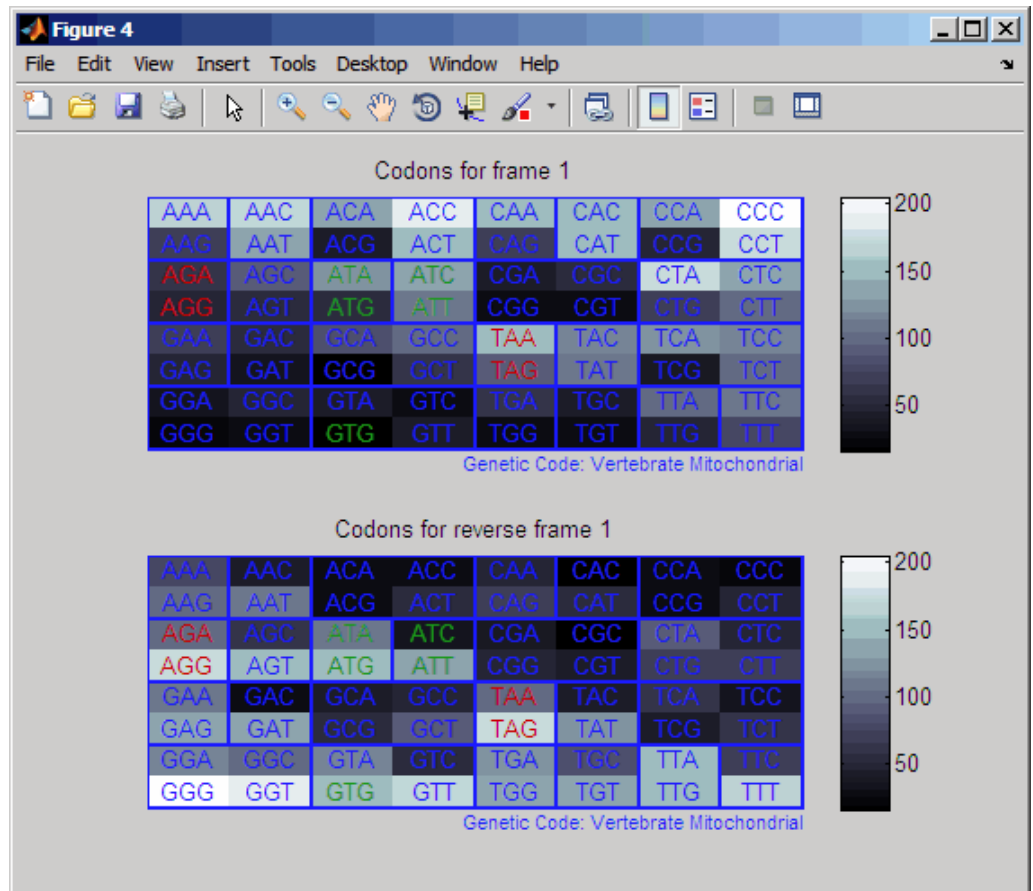
The codon counts for the first reading frame displays.

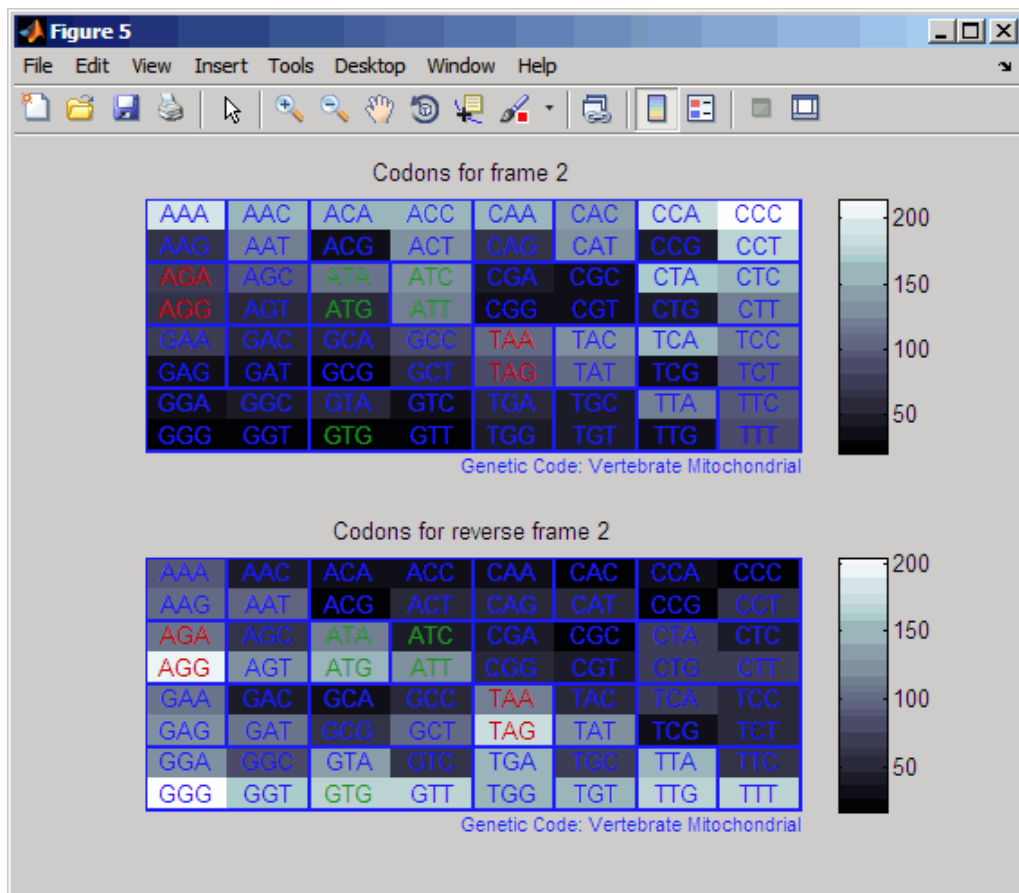
AAA - 167	AAC - 171	AAG - 71	AAT - 130
ACA - 137	ACC - 191	ACG - 42	ACT - 153
AGA - 59	AGC - 87	AGG - 51	AGT - 54
ATA - 126	ATC - 131	ATG - 55	ATT - 113
CAA - 146	CAC - 145	CAG - 68	CAT - 148
CCA - 141	CCC - 205	CCG - 49	CCT - 173
CGA - 40	CGC - 54	CGG - 29	CGT - 27
CTA - 175	CTC - 142	CTG - 74	CTT - 101
GAA - 67	GAC - 53	GAG - 49	GAT - 35
GCA - 81	GCC - 101	GCG - 16	GCT - 59
GGA - 36	GGC - 47	GGG - 23	GGT - 28
GTA - 43	GTC - 26	GTG - 18	GTT - 41
TAA - 157	TAC - 118	TAG - 94	TAT - 107
TCA - 125	TCC - 116	TCG - 37	TCT - 103
TGA - 64	TGC - 40	TGG - 29	TGT - 26
TTA - 96	TTC - 107	TTG - 47	TTT - 78

- 2 Count the codons in all six reading frames and plot the results in heat maps.

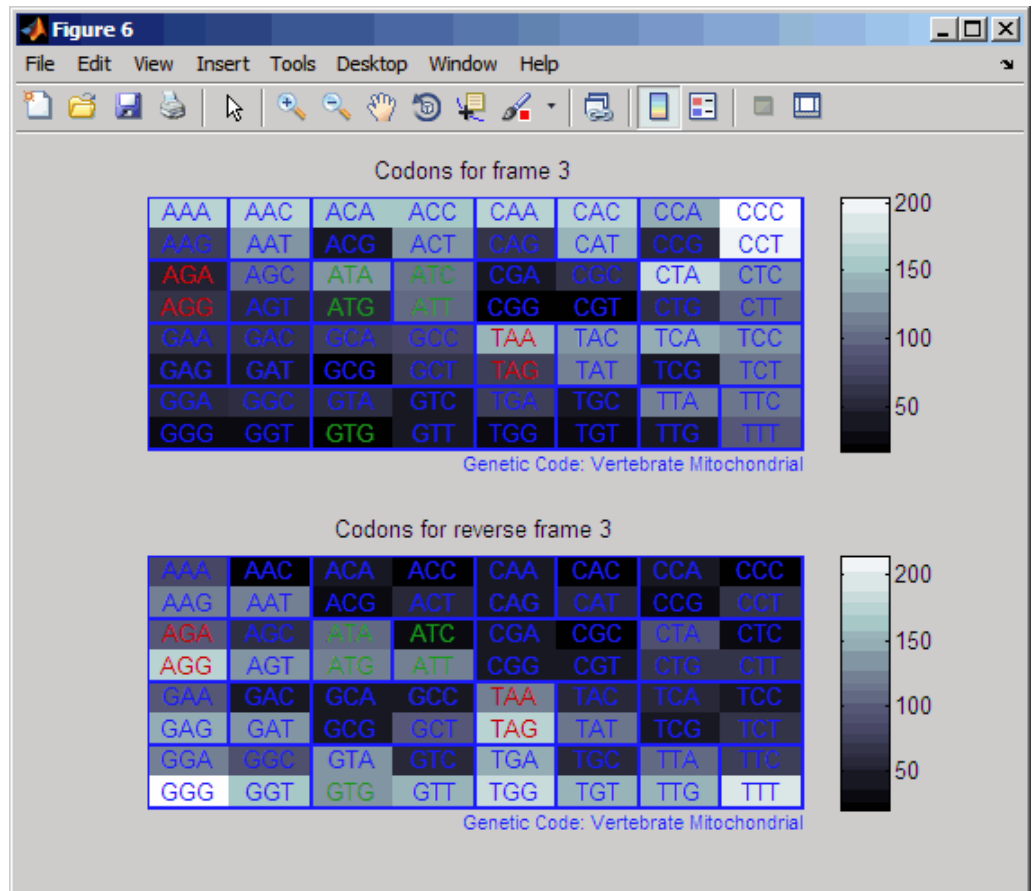
```
for frame = 1:3
    figure
    subplot(2,1,1);
    codoncount(mitochondria,'frame',frame,'figure',true,...
               'geneticcode','Vertebrate Mitochondrial');
    title(sprintf('Codons for frame %d',frame));
    subplot(2,1,2);
    codoncount(mitochondria,'reverse',true,'frame',frame,...
               'figure',true,'geneticcode','Vertebrate Mitochondrial');
    title(sprintf('Codons for reverse frame %d',frame));
end
```

Heat maps display all 64 codons in the 6 reading frames.









## Open Reading Frames

The following procedure illustrates how to locate the open reading frames using a specific genetic code. Determining the protein-coding sequence for a eukaryotic gene can be a difficult task because introns (noncoding sections) are mixed with exons. However, prokaryotic genes generally do not have introns and mRNA sequences have the introns removed. Identifying the start and stop codons for translation determines the protein-coding section, or open reading frame (ORF), in a sequence. Once you know the ORF for a gene or mRNA, you can translate a nucleotide sequence to its corresponding amino acid sequence.

After you read a sequence into the MATLAB environment, you can analyze the sequence for open reading frames. This procedure uses the human mitochondria genome as an example. See “Reading Sequence Information” on page 2-5.

- 1** Display open reading frames (ORFs) in a nucleotide sequence. In the MATLAB Command Window, type:

```
seqshoworfs(mitochondria);
```

If you compare this output to the genes shown on the NCBI page for NC\_012920, there are fewer genes than expected. This is because vertebrate mitochondria use a genetic code slightly different from the standard genetic code. For a list of genetic codes, see the “Genetic Code” table in the *aa2nt* reference page in the *Bioinformatics Toolbox Reference*.

- 2** Display ORFs using the Vertebrate Mitochondrial code.

```
orfs = seqshoworfs(mitochondria,...  
                  'GeneticCode','Vertebrate Mitochondrial',...  
                  'alternativestart',true);
```

Notice that there are now two large ORFs on the third reading frame. One starts at position 4470 and the other starts at 5904. These correspond to the genes ND2 (NADH dehydrogenase subunit 2 [Homo sapiens] ) and COX1 (cytochrome c oxidase subunit I) genes.

- 3** Find the corresponding stop codon. The start and stop positions for ORFs have the same indices as the start positions in the fields `Start` and `Stop`.

```
ND2Start = 4470;  
StartIndex = find(orfs(3).Start == ND2Start)  
ND2Stop = orfs(3).Stop(StartIndex)
```

The stop position displays.

```
ND2Stop =  
  
5511
```

- 4** Using the sequence indices for the start and stop of the gene, extract the subsequence from the sequence.

```
ND2Seq = mitochondria(ND2Start:ND2Stop)
```

The subsequence (protein-coding region) is stored in ND2Seq and displayed on the screen.

```
attaatcccctggcccaaccgctcatctactctaccatctttgcaggcac
actcatcacagcgtctaaagctcgactgattttttacctgagtaggcctag
aaataaacatgctagctttttattccagtcttaaccaaaaaataaacct
cgttccacagaagctgccatcaagtatttctcagcaagcaaccgcatc
cataatccttc . . .
```

- 5** Determine the codon distribution.

```
codoncount (ND2Seq)
```

The codon count shows a high amount of ACC, ATA, CTA, and ATC.

AAA - 10	AAC - 14	AAG - 2	AAT - 6
ACA - 11	ACC - 24	ACG - 3	ACT - 5
AGA - 0	AGC - 4	AGG - 0	AGT - 1
ATA - 23	ATC - 24	ATG - 1	ATT - 8
CAA - 8	CAC - 3	CAG - 2	CAT - 1
CCA - 4	CCC - 12	CCG - 2	CCT - 5
CGA - 0	CGC - 3	CGG - 0	CGT - 1
CTA - 26	CTC - 18	CTG - 4	CTT - 7
GAA - 5	GAC - 0	GAG - 1	GAT - 0
GCA - 8	GCC - 7	GCG - 1	GCT - 4
GGA - 5	GGC - 7	GGG - 0	GGT - 1
GTA - 3	GTC - 2	GTG - 0	GTT - 3
TAA - 0	TAC - 8	TAG - 0	TAT - 2
TCA - 7	TCC - 11	TCG - 1	TCT - 4
TGA - 10	TGC - 0	TGG - 1	TGT - 0
TTA - 8	TTC - 7	TTG - 1	TTT - 8

- 6** Look up the amino acids for codons ATA, CTA, ACC, and ATC.

```
aminolookup('code',nt2aa('ATA'))
aminolookup('code',nt2aa('CTA'))
```

```
aminolookup('code',nt2aa('ACC'))  
aminolookup('code',nt2aa('ATC'))
```

The following displays:

```
Ile isoleucine  
Leu leucine  
Thr threonine  
Ile isoleucine
```

## Amino Acid Conversion and Composition

The following procedure illustrates how to extract the protein-coding sequence from a gene sequence and convert it to the amino acid sequence for the protein. Determining the relative amino acid composition of a protein will give you a characteristic profile for the protein. Often, this profile is enough information to identify a protein. Using the amino acid composition, atomic composition, and molecular weight, you can also search public databases for similar proteins.

After you locate an open reading frame (ORF) in a gene, you can convert it to an amino sequence and determine its amino acid composition. This procedure uses the human mitochondria genome as an example. See “Open Reading Frames” on page 2-15.

- 1 Convert a nucleotide sequence to an amino acid sequence. In this example, only the protein-coding sequence between the start and stop codons is converted.

```
ND2AASeq = nt2aa(ND2Seq,'geneticcode',...  
                'Vertebrate Mitochondrial')
```

The sequence is converted using the `Vertebrate Mitochondrial` genetic code. Because the property `AlternativeStartCodons` is set to `'true'` by default, the first codon `att` is converted to `M` instead of `I`.

```
MNPLAQPVIYSTIFAGTLITALSSHWFFTWVGLEMNMLAFIPVLTKKMNP  
RSTEA AIKYFLTQATASMILLMAILFNNMLSGQWTMTNTTNQYSSLMIMM  
AMAMKLGMAPFHFVPEVTQGTPLTSGLLLLTWQKLAPISIMYQISPSLN  
VLLLLTLSILSIMAGSWGGLNQTQLRKILAYSSITHMGWMMAVLPYNPNM  
TILNLTIIYIILTTTAFLLLNLSSTTTLLLSRTWNKLTWLTPLIPSTLLS
```

```
LGGLPPLTGFLPKWAIIEEFTKNNSLIPTIMATITLLNLYFYLRRIYST  
SITLLPMSNNVKKMKWQFEHTKPTPFLPTLIALTLLLPISPFMLMIL
```

- 2** Compare your conversion with the published conversion in the GenPept database.

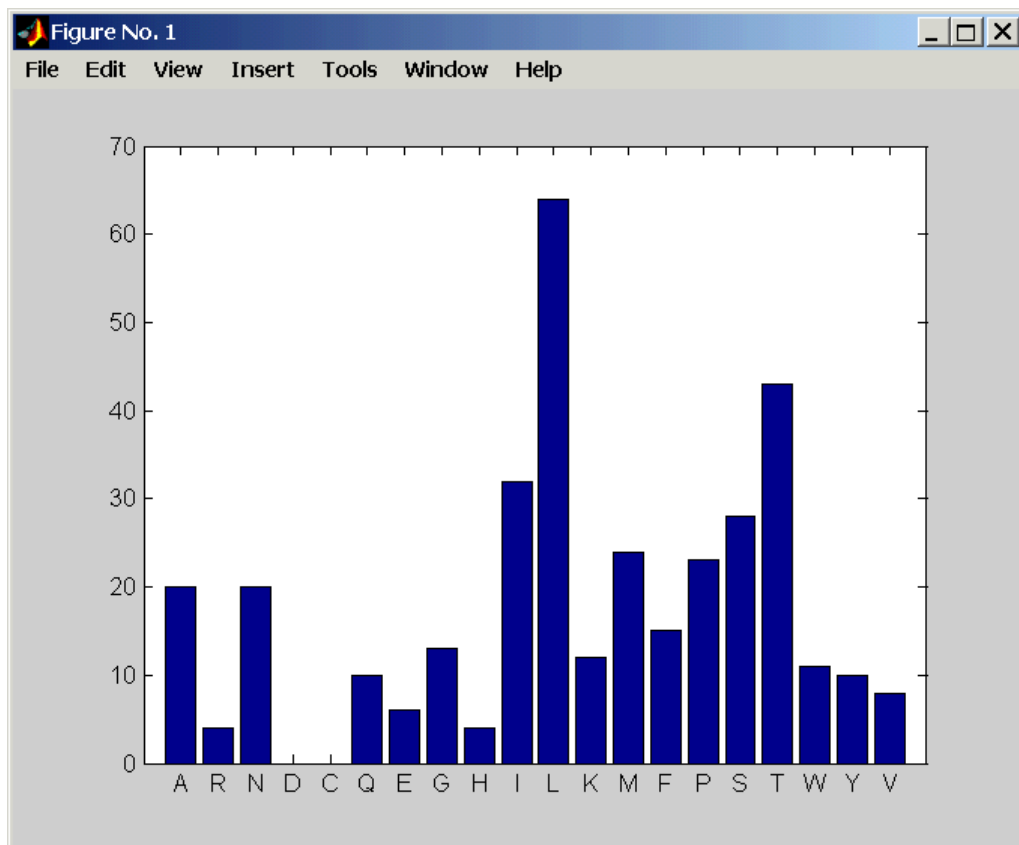
```
ND2protein = getgenpept('YP_003024027','sequenceonly',true)
```

The `getgenpept` function retrieves the published conversion from the NCBI database and reads it into the MATLAB Workspace.

- 3** Count the amino acids in the protein sequence.

```
aaccount(ND2AASeq, 'chart', 'bar')
```

A bar graph displays. Notice the high content for leucine, threonine and isoleucine, and also notice the lack of cysteine and aspartic acid.



4 Determine the atomic composition and molecular weight of the protein.

```
atomiccomp(ND2AASeq)
molweight (ND2AASeq)
```

The following displays in the MATLAB Workspace:

```
ans =
    C: 1818
    H: 2882
    N: 420
    O: 471
```

S: 25

ans =

3.8960e+004

If this sequence was unknown, you could use this information to identify the protein by comparing it with the atomic composition of other proteins in a database.

## Example: Sequence Alignment

In this section...
“Overview of Example” on page 2-22
“Finding a Model Organism to Study” on page 2-22
“Retrieving Sequence Information from a Public Database” on page 2-24
“Searching a Public Database for Related Genes” on page 2-27
“Locating Protein Coding Sequences” on page 2-29
“Comparing Amino Acid Sequences” on page 2-33

### Overview of Example

Determining the similarity between two sequences is a common task in computational biology. Starting with a nucleotide sequence for a human gene, this example uses alignment algorithms to locate and verify a corresponding gene in a model organism.

### Finding a Model Organism to Study

The following procedure illustrates how to use the MATLAB Help browser to search the Web for information. In this example, you are interested in studying Tay-Sachs disease. Tay-Sachs is an autosomal recessive disease caused by the absence of the enzyme beta-hexosaminidase A (Hex A). This enzyme is responsible for the breakdown of gangliosides (GM2) in brain and nerve cells.

First, research information about Tay-Sachs and the enzyme that is associated with this disease, then find the nucleotide sequence for the human gene that codes for the enzyme, and finally find a corresponding gene in another organism to use as a model for study.

- 1 Use the MATLAB Help browser to explore the Web. In the MATLAB Command window, type

```
web('http://www.ncbi.nlm.nih.gov/')
```



The MATLAB Help browser opens with the home page for the NCBI Web site.

- 2 Search the NCBI Web site for information. For example, to search for Tay-Sachs, from the **Search** list, select **NCBI Web Site**, and in the **for** box, enter **Tay-Sachs**.

The screenshot shows the top of the NCBI website. The header includes the NCBI logo and the text "National Center for Biotechnology Information", "National Library of Medicine", and "National Institutes of Health". Below the header is a navigation bar with links for PubMed, Entrez, BLAST, OMIM, Books, TaxBrowser, and Structure. A search box is present with a dropdown menu set to "NCBI Web Site" and the text "Tay-Sachs" entered in the "for" field. A "Go" button is to the right of the search box.

The NCBI Web search returns a list of links to relevant pages.

The screenshot shows the search results page. The header includes the NCBI logo and the text "NCBI Web Site Search". Below the header is a navigation bar with links for PubMed, Nucleotide, Protein, Genome, Structure, Popset, and Taxonomy. A search box is present with a dropdown menu set to "NCBI Web Site" and the text "Tay-Sachs" entered in the "for" field. A "Go" button and a "Clear" button are to the right of the search box. Below the search box are links for Limits, Preview/Index, History, Clipboard, and Details. A "Display" dropdown menu is set to "Summary", and a "Show:" dropdown menu is set to "20". A "Send to" dropdown menu is set to "Text". The first result is "1: Tay-Sachs Disease" with a "Related sites" link. The description of the result is: "Genes and disease provides short descriptions of inherited disorders. It is hosted by the National Center for Biotechnology Information (NCBI), a division of the National Library of Medicine (NLM) at the National Institutes of Health (NIH). Category: [product-service.information.article](#)".


- 3 Select a result page. For example, click the link labeled **Tay-Sachs Disease**.

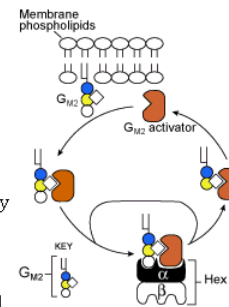
A page in the genes and diseases section of the NCBI Web site opens. This section provides a comprehensive introduction to medical genetics. In particular, this page contains an introduction and pictorial representation of the enzyme Hex A and its role in the metabolism of the lipid GM2 ganglioside.



**genes and disease**  
from the national center for biotechnology information  
NCBI

Short Contents | Full Contents | Other books @ NCBI

Navigation	<i>Genes and Disease</i> →  <u>Nutritional and Metabolic Diseases</u>
<u><i>About this book</i></u>	
<u>Nutritional and Metabolic Diseases</u>	<b>Tay-Sachs disease</b>
<u>Adrenoleukodystrophy</u>	Tay-Sachs disease, a heritable metabolic disorder commonly associated with Ashkenazi Jews, has also been found in the French Canadians of Southeastern Quebec, the Cajuns of Southwest Louisiana, and other populations throughout the world. The severity of expression and the age at onset of Tay-Sachs varies from infantile and juvenile forms that exhibit paralysis, dementia, blindness and
<u>Diabetes, type 1</u>	
<u>Gaucher disease</u>	
<u>Glucose galactose malabsorption</u>	
<u>Hereditary hemochromatosis</u>	
<u>Maple syrup urine disease</u>	
<u>Menkes syndrome</u>	



#### 4 After completing your research, you have concluded the following:

The gene *HEXA* codes for the alpha subunit of the dimer enzyme hexosaminidase A (Hex A), while the gene *HEXB* codes for the beta subunit of the enzyme. A third gene, *GM2A*, codes for the activator protein GM2. However, it is a mutation in the gene *HEXA* that causes Tay-Sachs.

## Retrieving Sequence Information from a Public Database

The following procedure illustrates how to find the nucleotide sequence for a human gene in a public database and read the sequence information into the MATLAB environment. Many public databases for nucleotide sequences (for example, GenBank, EMBL-EBI) are accessible from the Web. The MATLAB Command Window with the MATLAB Help browser provide an integrated environment for searching the Web and bringing sequence information into the MATLAB environment.

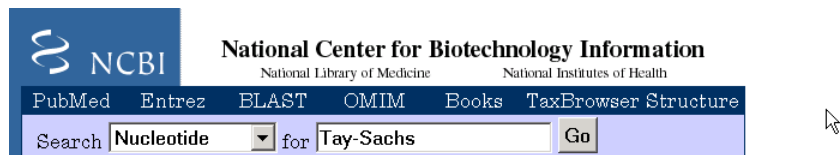
After you locate a sequence, you need to move the sequence data into the MATLAB Workspace.

- 1 Open the MATLAB Help browser to the NCBI Web site. In the MATLAB Command Window, type

```
web('http://www.ncbi.nlm.nih.gov/')
```

The MATLAB Help browser window opens with the NCBI home page.

- 2 Search for the gene you are interested in studying. For example, from the **Search** list, select **Nucleotide**, and in the **for** box enter Tay-Sachs.



The search returns entries for the genes that code the alpha and beta subunits of the enzyme hexosaminidase A (Hex A), and the gene that codes the activator enzyme. The NCBI reference for the human gene HEXA has accession number NM\_000520.

The screenshot shows the NCBI Nucleotide search interface. The search term 'Tay-Sachs' is entered in the search box. The results are displayed in a table with three entries:

Item	Accession	Description	Links
1:	<a href="#">NM_000405</a>	Homo sapiens GM2 ganglioside activator protein (GM2A), mRNA gi 16507969 ref NM_000405.2 [16507969]	<a href="#">Links</a>
2:	<a href="#">NM_000521</a>	Homo sapiens hexosaminidase B (beta polypeptide) (HEXB), mRNA gi 13128866 ref NM_000521.2 [13128866]	<a href="#">Links</a>
3:	<a href="#">NM_000520</a>	Homo sapiens hexosaminidase A (alpha polypeptide) (HEXA), mRNA gi 13128865 ref NM_000520.2 [13128865]	<a href="#">Links</a>

- 3** Get sequence data into the MATLAB environment. For example, to get sequence information for the human gene HEXA, type

```
humanHEXA = getgenbank('NM_000520')
```

---

**Note** Blank spaces in GenBank accession numbers use the underline character. Entering 'NM 00520' returns the wrong entry.

---

The human gene is loaded into the MATLAB Workspace as a structure.

```
humanHEXA =
    LocusName: 'NM_000520'
    LocusSequenceLength: '2255'
    LocusNumberofStrands: ''
    LocusTopology: 'linear'
```

```

LocusMoleculeType: 'mRNA'
LocusGenBankDivision: 'PRI'
LocusModificationDate: '13-AUG-2006'
Definition: 'Homo sapiens hexosaminidase A (alpha polypeptide) (HEXA), mRNA.'
Accession: 'NM_000520'
Version: 'NM_000520.2'
GI: '13128865'
Project: []
Keywords: []
Segment: []
Source: 'Homo sapiens (human)'
SourceOrganism: [4x65 char]
Reference: {1x58 cell}
Comment: [15x67 char]
Features: [74x74 char]
CDS: [1x1 struct]
Sequence: [1x2255 char]
SearchURL: [1x108 char]
RetrieveURL: [1x97 char]

```

## Searching a Public Database for Related Genes

The following procedure illustrates how to find the nucleotide sequence for a mouse gene related to a human gene, and read the sequence information into the MATLAB environment. The sequence and function of many genes is conserved during the evolution of species through homologous genes. Homologous genes are genes that have a common ancestor and similar sequences. One goal of searching a public database is to find similar genes. If you are able to locate a sequence in a database that is similar to your unknown gene or protein, it is likely that the function and characteristics of the known and unknown genes are the same.

After finding the nucleotide sequence for a human gene, you can do a BLAST search or search in the genome of another organism for the corresponding gene. This procedure uses the mouse genome as an example.

- 1 Open the MATLAB Help browser to the NCBI Web site. In the MATLAB Command window, type

```
web('http://www.ncbi.nlm.nih.gov')
```

- 2 Search the nucleotide database for the gene or protein you are interested in studying. For example, from the **Search** list, select **Nucleotide**, and in the **for** box enter **hexosaminidase A**.

The screenshot shows the NCBI search page. At the top, it says "National Center for Biotechnology Information" with "National Library of Medicine" and "National Institutes of Health" below it. There are navigation links for PubMed, Entrez, BLAST, OMIM, Books, TaxBrowser, and Structure. The search bar contains "Nucleotide" in a dropdown menu, followed by "for" and "hexosaminidase A" in a text box, and a "Go" button.

The search returns entries for the mouse and human genomes. The NCBI reference for the mouse gene **HEXA** has accession number **AK080777**.

The screenshot shows the NCBI search results page. At the top, it says "National Center for Biotechnology Information" with "National Library of Medicine" and "National Institutes of Health" below it. There are navigation links for PubMed, Nucleotide, Protein, Genome, Structure, PMC, Taxonomy, OMIM, and Books. The search bar contains "Nucleotide" in a dropdown menu, followed by "for" and "hexosaminidase A" in a text box, and "Go" and "Clear" buttons. Below the search bar are links for Limits, Preview/Index, History, Clipboard, and Details. There are also "Display" and "Summary" dropdown menus, a "Show" dropdown menu set to "20", and a "Send to" dropdown menu set to "Text". Below these are "Items 1-16 of 16" and "One page." The first result is listed as "1: [AK080777](#)" with a "Links" link to its right. The description for this result is: "Mus musculus 9.5 days embryo parthenogenote cDNA, RIKEN full-length enriched library, clone:B130019N09 product:hexosaminidase A, full insert sequence gi|26348756| dbj|AK080777.1|[26348756]".

- 3 Get sequence information for the mouse gene into the MATLAB environment. Type

```
mouseHEXA = getgenbank('AK080777')
```

The mouse gene sequence is loaded into the MATLAB Workspace as a structure.

```

mouseHEXA =

        LocusName: 'AK080777'
    LocusSequenceLength: '1839'
    LocusNumberofStrands: ''
        LocusTopology: 'linear'
    LocusMoleculeType: 'mRNA'
    LocusGenBankDivision: 'HTC'
    LocusModificationDate: '02-SEP-2005'
        Definition: [1x150 char]
        Accession: 'AK080777'
        Version: 'AK080777.1'
            GI: '26348756'
        Project: []
        Keywords: 'HTC; CAP trapper.'
        Segment: []
        Source: 'Mus musculus (house mouse)'
    SourceOrganism: [4x65 char]
    Reference: {1x8 cell}
    Comment: [8x66 char]
    Features: [33x74 char]
        CDS: [1x1 struct]
    Sequence: [1x1839 char]
    SearchURL: [1x107 char]
    RetrieveURL: [1x97 char]

```

## Locating Protein Coding Sequences

The following procedure illustrates how to convert a sequence from nucleotides to amino acids and identify the open reading frames. A nucleotide sequence includes regulatory sequences before and after the protein coding section. By analyzing this sequence, you can determine the nucleotides that code for the amino acids in the final protein.

After you have a list of genes you are interested in studying, you can determine the protein coding sequences. This procedure uses the human gene HEXA and mouse gene HEXA as an example.

- 1 If you did not retrieve gene data from the Web, you can load example data from a MAT-file included with the Bioinformatics Toolbox software. In the MATLAB Command window, type

```
load hexosaminidase
```

The structures `humanHEXA` and `mouseHEXA` load into the MATLAB Workspace.

- 2 Locate open reading frames (ORFs) in the human gene. For example, for the human gene `HEXA`, type

```
humanORFs = seqshoworfs(humanHEXA.Sequence)
```

`seqshoworfs` creates the output structure `humanORFs`. This structure contains the position of the start and stop codons for all open reading frames (ORFs) on each reading frame.

```
humanORFs =
```

```
1x3 struct array with fields:  
  Start  
  Stop
```

The Help browser opens displaying the three reading frames with the ORFs colored blue, red, and green. Notice that the longest ORF is in the first reading frame.



## Frame 1

```

000001 agttgccgacgccccggcacaatccgctgcacgtagcaggagcctcagggtccaggccgggaagtga
000065 aagggcagggtgtgggtcctcctggggctgcaggcgcagagccgctctggtcacgtgattcgc
000129 cgataagtcacggggggcgcgctcacctgaccagggtctcacgtggccagccccctccgagagg
000193 ggagaccagcggggccatgacaagctccaggctttgggttttgcgtgtgctggcggcagcgtctg
000257 caggacgggcgacggccctctggccctggcctcagaacttccaaacctccgaccagcgtacgt
000321 cctttaccogaacaactttcaatccagtaacgatgtoagctcggccgcgcagcccggtctca
000385 gtccctgacgaggccttccagcgtatcgtgacctgcttttgggttcgggtcttggcccgctc
000449 cttacctcacagggaaacggcatacaactgggagaagaatgtgtgggtgtctctgtagtcacacc
000513 tggatgtaaccagcttccactttggagtcagtgaggagaattataccctgaccataaatgatgac
000577 cagtgtttactcctctctgagactgtctggggagctctccgaggtctggagacttttagccagc
000641 ttgtttgaaaatctgctgagggcacattctttatcaacaagactgagattgaggactttccccg
000705 ctttctcaccggggcttgcgtgtggatacatctgcaccattacctgccactctctagcactctg
000769 gacactctggatgtcatggcgtacaataaattgaaactgttccactggcactctggtagatgac
000833 ctctctcccatatgagagcttcaactttccagagctcactgagaaaggggtcctacaacctgt
000897 caccacatctacacagcagcaggtatgaaaggaggtcaattgaaacgcagcgtccggggatc
000961 cgtgtgcttgacagatttgacactcctggccacactttgtcctggggaccagggtatccctggat
001025 tactgactccttgcactctgggtctgagccctctggcaccctttggaccagtgaaatccagctc
001089 caataatacctatgagttcatgagcacaattctcttagaagtcagctctgtcttccagatttt
001153 tatctcatcttggaggagatgaggttgatttccactgctggaagtccaaccagagatccagg
001217 actttatgaggaagaaggcttoggtaggacttcaagcagctggagctctctacatccagac
001281 gctgtggacatcgtctctctcttatggcaagggtatgtgtgtggcaggaggttttgataat
001345 aaagtaaagattcagccagacacaatcatacagggtgtggcagagagatattccagtgaaacta
001409 tgaaggagctggaactggtcaccaaggccggctccgggcccctctctctgcccctggtaact
001473 gaaccgtatatacctatggccctgactggaaggatttctacatagtggaaccctggcatttgaa
001537 ggtaccctgagcagaaggctctgggtgattggtggagaggcttgatgtggggagaatagtgg
001601 acaacacaaacctgggtccccaggctctggcccagagcaggggctgttgccgaaaggctgtggag
001665 caacaagttgacatctgacctgacatttgccatgaaactgttgcacttccgctgtgaaattg
001729 ctgaggcagggtgtccaggcccaaccctcaatgtaggctctctgtgagcaggagtttgaacaga
001793 cctgagccccaggcaccgaggagggtgctggctgtagggtgaatggtagtgagccaggctcca
001857 ctgcatcctggccaggggacggagcccttgccctcgtgccccttgccctgctgcccctgtgct
001921 tggagagaaggggccgggtgctggcgtcgcattcaataaaagagtaatgtggcattttctata
001985 ataaacatggattacctgtgtttaaaaaaaaaagtgtgaaatggcgttaggtaagggcacagcc
002049 aggtggagtcagtgctgcccctgaggtcttttaagttgagggctgggaaatgaaacctatagc
002113 ctttgtgctgttotgccttgctgtgagctatgtcactccccctcccactcctgaccatattcca
002177 gacacctgccctaatacctcagcctgctcacttcaactctgcaattatctccaaggcgttggtta
002241 tatggaaaagatgtaggggcttggaggtgtctggaacagtggggagggctccagaccacact
002305 ggtcacagaagagcctctccccatgcatactcactccactccctcccctagagctattctcct
002369 ttgggtttottgctgcttcaattttatacaaccattatthaaatattatthaaacacatttgtt
002433 ctcta

```

**3** Locate open reading frames (ORFs) in the mouse gene. Type:

```
mouseORFs = seqshoworfs(mouseHEXA.Sequence)
```

seqshoworfs creates the structure mouseORFS.

```
mouseORFs =
```

```
1x3 struct array with fields:
```

```
Start
```

```
Stop
```

The mouse gene shows the longest ORF on the first reading frame.

```
Frame 1
```

```
000001 gctgctggaaggggagctggccgggtgggccatggccggctgcaggctctgggtttcgctgctgc
000065 tggcggcggcgttggcttgccttggccacggcactgtggccgtggcccagtaacatccaaaccta
000129 ccaccggcgtacaccctgtaccccaacaacttccagttccggtaaccatgtcagttcggcccg
000193 caggcgggctgcgtcgtcctgcacgagggccttccagcctaccgtaacctgctctcgggtccg
000257 gctcttggccccgaccagcttctcaaataaacagcaaacgttgggaagaacattctgggtggt
000321 ctccgtcgtcacagctgaatgtaatgaatttctaatttggagtcggtagaaaaatcacaccta
000385 accattaatgatgaccagtggttactcgcctctgagactgtctggggcgtctccgaggtctgg
000449 agacttccagtcagcttgttggaaatcagctgagggcacgttctttatcaacaagacaagat
000513 taaagacttctcctcattccctcaccggggcgtactgctggatacatctcgccattacctgca
000577 ttgtctagcactcctggatcacctggatgtcatggcatacaataaatcaacctggtccactggc
000641 acttggtygacgactcttctctcccatatgagagcttcaacttcccagagctcaccagaaaaggg
000705 gtcctcaacctgtcactcacatctacacagcacaggatgtgaaggaggtcatgaaacgca
000769 aggcctcggggtatccgtgctggcagaatttgacactcctggccacactttgtcctgggggc
000833 cagggtgccctgggttatlaaacacttgcactctgggtctcatctctctggcaactttggacc
000897 ggtgaaccccagctctcaacagcactatgacttcatgagcacactctcctggagatcagctca
000961 gctctcccggacttttatctccactgggaggggatgaagtcgactcactcgtggaagtcca
001025 accccaacatccaggccttcatgaagaaaaagggcttactgactcaagcagctggagctctt
001089 ctacatccagacgctgctggacatcgtctctgatattgacaagggctatgtggtgtggcaggag
001153 gtatttgataataaagtgaaggttcggccagatacaatcatacaggtgtggcgggaagaaatgc
001217 cagtagagtacatgttgagatgaagatataccagggtggctccgggcccctgctgctcgc
001281 tccctggtacctgaacctgtaaagtatggccctgactggaaggacatgtacaagtgagagccc
001345 ctggcgtttcatggtacgcctgaacagaaggtctggctcatggaggggaggcctgtatgtggg
001409 gagagtatgtggacagcacaacctggctcccagactctggcccagagcgggtgcccctcgtga
001473 gagactgtggagcagtaacctgacaactaatatagactttgcctttaaactgtgtgcgatttc
001537 cgttgtgagctggtgaggagaggaatccagggccagcccacaggtgtaggctcgtgtgagcagg
001601 agtttgagcagacttgagccaccagtgctgaacacccaggaggttgctgtcctttgagtcagct
001665 gcgctgagcaccagggaggtgctggcccttaagagagcaggtcccggggcaggggctaactcttc
001729 actgctcccggccaggggagagcacccttgcccgtgtgcccctgtgactacagagaaggagg
001793 ctggtgctggcactgggtgttcaataaagatctatgtggcattttctc
```

## Comparing Amino Acid Sequences

The following procedure illustrates how to use global and local alignment functions to compare two amino acid sequences. You could use alignment functions to look for similarities between two nucleotide sequences, but alignment functions return more biologically meaningful results when you are using amino acid sequences.

After you have located the open reading frames on your nucleotide sequences, you can convert the protein coding sections of the nucleotide sequences to their corresponding amino acid sequences, and then you can compare them for similarities.

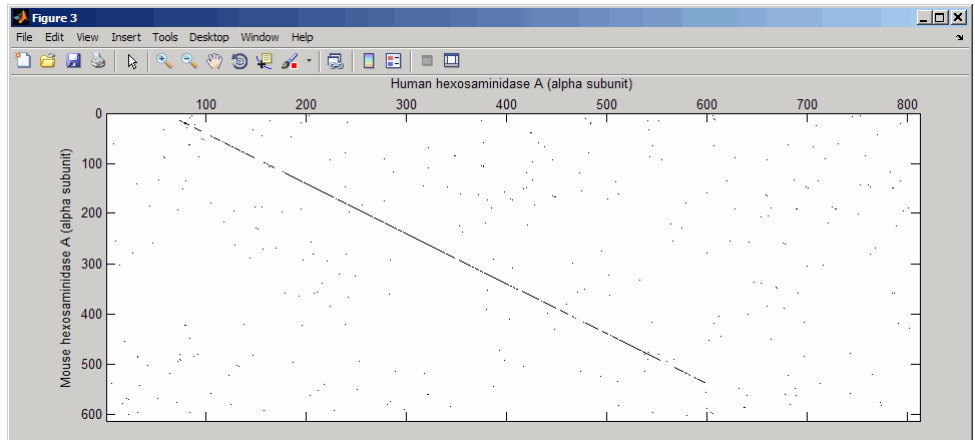
- 1 Using the open reading frames identified previously, convert the human and mouse DNA sequences to the amino acid sequences. Because both the human and mouse HEXA genes were in the first reading frames (default), you do not need to indicate which frame. Type

```
humanProtein = nt2aa(humanHEXA.Sequence);  
mouseProtein = nt2aa(mouseHEXA.Sequence);
```

- 2 Draw a dot plot comparing the human and mouse amino acid sequences. Type

```
seqdotplot(mouseProtein,humanProtein,4,3)  
ylabel('Mouse hexosaminidase A (alpha subunit)')  
xlabel('Human hexosaminidase A (alpha subunit)')
```

Dot plots are one of the easiest ways to look for similarity between sequences. The diagonal line shown below indicates that there may be a good alignment between the two sequences.



- 3** Globally align the two amino acid sequences, using the Needleman-Wunsch algorithm. Type

```
[GlobalScore, GlobalAlignment] = nwalignment(humanProtein,...  
                                              mouseProtein);  
showalignment(GlobalAlignment)
```

`showalignment` displays the global alignment of the two sequences in the Help browser. Notice that the calculated identity between the two sequences is 60%.

```

Identities = 491/812 (60%), Positives = 575/812 (71%)
001  SCRRPAQSAARSRLRSRPEVKGQGVGPPGVAGAEPLVLT*FADKSRGRSPDQGLTWPAAPER
      ||           |           |           |           |           |
001  -----AA-----GR-----G-----A---G-R-----W-----

065  GDQRAMTSSRLWFSLLLAAAFAGRATALWPPONFOTSDORYVLYPNNFQFOYDVSSAAOPGCS
      ||::| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
010  ----AMAGCRLWVSLLLAAALACLATALWPPQYIQTYHRRYTLYPNNFQFRYHVSSAAQAGCV

129  VLDEAFQRYRDLLFGSGSWPRPYLTGKRHTLEKNVLVSVVTPGCNQLPTLESVENYTLTINDD
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
070  VLDEAFRRYRNLLFGSGSWPRPSFSNKQOTLGKNILVSVVTAECEFPNLESVENYTLTINDD

193  OCLLLSETVWGALRGLETFSQLVWKSAGETFFINKTEIEDFPRFPHRGLLLDTSRHYLPLSSIL
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
134  OCLLASETVWGALRGLETFSQLVWKSAGETFFINKTKIKDFPRFPHRGVLLDTSRHYLPLSSIL

257  DTLDVMAYNKLVNFHHLVDDPSFPYESFTFPELMRKGSYNPVTTHIYTAQDVKEVIEYARLRGI
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
198  DTLDVMAYNKFNVFHHLVDDSSFPYESFTFPELTRKGSFNPVTTHIYTAQDVKEVIEYARLRGI

321  RVLAEFDTPGHTLSWGPPIGLLTPCYSGSEPSGTFGPVNPSLNNTYEFMSTFFLEVSSVFPDF
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
262  RVLAEFDTPGHTLSWGPAPGLLTPCYSGSHLSGTFGPVNPSLNSTYDFMSTLFLEISSVFPDF

385  YLHLGGDEVDFTCWKSNPFIQDFMRKKGFGEDFKQLESFYIQTLDDIVSSYGKGYVVWQEVFDN
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
326  YLHLGGDEVDFTCWKSNPNIQAFMKKKGF-IDFKQLESFYIQTLDDIVSDYDKGYVVWQEVFDN

449  KVKIQPDTIIQVWREDIPVNYMKELELVTKAGFRALLSAPWYLNRIISYGPDWKDFYIVEPLAFE
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
389  KVKVRPDTIIQVWREEMPVEYMLEMODITRAGFRALLSAPWYLNRVKYPDWKDMYKVEPLAFH

513  GTPEQKALVIGGEACMWGEYVDNINLVPRLWPRAGAVAERLWSNKLTSDLTFAYERLSHFRCLE
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
453  GTPEQKALVIGGEACMWGEYVDSINLVPRLWPRAGAVAERLWSNLTINI DFAFKRLSHFRCELE

577  LRRGVQAOPLNVGFCEQEFQOT*APGTEEGAGCR*MVVEPGFHCILARGRSPLPSCPLPACPCA
      : | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
517  VRRGIQAQPI SVGCCEQEFQOT*A--T--SA--E---HPG-----G-----C---CP--

641  WRERGRNRSHSIKSNVAFFYNKHGLPVFKKSVNGVRVRAQPWSQCLPLRSFKLRAGNETYS
      | : : | | | | | | | | | | | | | | | | | | | | | | | | | | | |
552  -----L-SQ-LR--*A-----P--RR-V--LALR-E---Q-VP--G-Q--G-*SFT

705  LCAVLPCI*AMSLPSHS*PYSRHLP*SSACSLHFCIISPRRWYMEKDVGAWRCSGQWGGLQTOP
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
578  -----A-SRPGES--T---P---CP---C--APVT--TEKEAGA---GT--GV--Q-

769  GHRRASPPCILIHLPPELEFSFGFLAASILYNHYLNIKHLIFS
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
606  --*R-----S-MW-HF-----L--

```

The alignment is very good between amino acid position 69 and 599, after which the two sequences appear to be unrelated. Notice that there is a stop (\*) in the sequence at this point. If you shorten the sequences to include only the amino acids that are in the protein you might get a better alignment. Include the amino acid positions from the first methionine (M) to the first stop (\*) that occurs after the first methionine.

- 4 Trim the sequence from the first start amino acid (usually M) to the first stop (\*) and then try alignment again. Find the indices for the stops in the sequences.

```
humanStops = find(humanProtein == '*')
```

```
humanStops =
```

```
    41    599    611    713    722    730
```

```
mouseStops = find(mouseProtein == '*')
```

```
mouseStops =
```

```
    539    557    574    606
```

Looking at the amino acid sequence for `humanProtein`, the first M is at position 70, and the first stop after that position is actually the second stop in the sequence (position 599). Looking at the amino acid sequence for `mouseProtein`, the first M is at position 11, and the first stop after that position is the first stop in the sequence (position 557).

- 5 Truncate the sequences to include only amino acids in the protein and the stop.

```
humanProteinORF = humanProtein(70:humanStops(2))
```

```
humanProteinORF =
```

```
MTSSRLWFSLLLAFAFRATLWPWPQNFQTSQDRYVLYPNNFQFQYDV  
SSAAQPGCSVLDEAFQRYRDLLFGSGSWPRPYLTGKRHTLEKNVLVSVV  
TPGCNQLPTLESVENYTLTINDDQCLLLSETVWGALRGLETFSQLVWKSA  
EGTFFINKTEIEDFPRFPHRGLLLDTSRHYLPLSSILDVLMAYNKLNV
```

```

FWHHLVDDPSFPYESFTFPELMRKGSYNPVTHIYTAQDVKEVIEYARLRG
IRVLAEFDTPGHTLSWGP GIPGLLTPCYSGSEPSGTFGPVNP SLNNTYEF
MSTFFLEVSSVFPDFYLHLGGDEVDFTCWKS NPEIQDFMRKKGFGE DFKQ
LESFYIQTLLDIVSSYGKGYVVWQEVFDNKVKIQPDTIIQVWREDIPVNY
MKELELVTKAGFRALLSAPWYLNRI SYGPDWKDFYIVEPLAFEGTPEQKA
LVIGGEACMWGEYVDNTNLV PRLWPRAGAVAERLWSNKL TSDLT FAYERL
SHFRCELLRRGVQAQPLNVGFCEQEFEQT*

```

```
mouseProteinORF = mouseProtein(11:mouseStops(1))
```

```
mouseProteinORF =
```

```

MAGCRLWVSLLLAAALACLATALWPWPQYI QTYHRRYTLYPNNFQFRYHV
SSAAQAGCVVLDEAFRRYRNLLFGSGSWPRPSFSNKQQLGKNILVVSVV
TAECEFPNLESVENYTLTINDDQCLLASETVW GALRGLTFSQLVWKSA
EGTFFINKTKIKDFPRFPHRGVLLDTSRHYLPLSSILD TDMAYNKFNV
FWHHLVDDSSFPYESFTFPELTRKGSFNPVTHIYTAQDVKEVIEYARLRG
IRVLAEFDTPGHTLSWGP GAGPLLTPCYSGSHLSGTFGPVNP SLNSTYDF
MSTLFLEISSVFPDFYLHLGGDEVDFTCWKS NPNIAQAFMKKKGF TDFKQL
ESFYIQTLLDIVSDYDKGYVVWQEVFDNKVKVRPDTIIQVWREEMPVEYM
LEMQDITRAGFRALLSAPWYLN RVKYGPDWKDMYKVEPLAFHG TPEQKAL
VIGGEACMWGEYVDSTNLV PRLWPRAGAVAERLWSSNL TTNIDFAFKRLS
HFRCELVRRGIQAQPISVGCCEQEFEQT*

```

**6** Globally align the trimmed amino acid sequences. Type

```

[GlobalScore_trim, GlobalAlignment_trim] = nwalign(humanProteinORF,...
                                                    mouseProteinORF);
showalignment(GlobalAlignment_trim)

```

`showalignment` displays the results for the second global alignment. Notice that the percent identity for the untrimmed sequences is 60% and 84% for trimmed sequences.





the `seqshoworfs` function. Remember that the ORF for the human HEXA gene and the ORF for the mouse HEXA were both on the first reading frame.

```
humanORFs = seqshoworfs(humanHEXA.Sequence)
```

```
humanORFs =
```

```
1x3 struct array with fields:
```

```
    Start
```

```
    Stop
```

```
mouseORFs = seqshoworfs(mouseHEXA.Sequence)
```

```
mouseORFs =
```

```
1x3 struct array with fields:
```

```
    Start
```

```
    Stop
```

```
humanPORF = nt2aa(humanHEXA.Sequence(humanORFs(1).Start(1):...
                                     humanORFs(1).Stop(1)));
```

```
mousePORF = nt2aa(mouseHEXA.Sequence(mouseORFs(1).Start(1):...
                                       mouseORFs(1).Stop(1)));
```

```
[GlobalScore2, GlobalAlignment2] = nwalignment(humanPORF, mousePORF);
```

Show the alignment in the Help browser.

```
showalignment(GlobalAlignment2)
```

The result from first truncating a nucleotide sequence before converting it to an amino acid sequence is the same as the result from truncating the amino acid sequence after conversion. See the result in step 6.

An alternative method to working with subsequences is to use a local alignment function with the nontruncated sequences.

- 8 Locally align the two amino acid sequences using a Smith-Waterman algorithm. Type

```
[LocalScore, LocalAlignment] = swalign(humanProtein,...  
                                         mouseProtein)
```

```
LocalScore =  
          1057
```

```
LocalAlignment =
```

```
RGDQR-AMTSSRLWFSLLLAAAFAGRATALWPWPQNFQTS DQRYV . . .  
|| | ||:: ||| |||||:| ||||| | :|| :||: . . .  
RGAGRWAMAGCRLWVSLLLAAALACLATALWPWPQYIQTYHRRYT . . .
```

- 9 Show the alignment in color.

```
showalignment(LocalAlignment)
```

```
Identities = 454/547 (83%), Positives = 514/547 (94%)
 1  RGDQR-AMTSSRLWFSLLLAFAAGRATALWPWPQNFTQSDQRYVLYPNNFQFYDVSSAAQPG
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 1  RGAGRWAMAGCRLWVSLLLAAALACLATALWPWPQYIQTYHRRYTLYPNNFQFRYHVSSAAQAG

64  CSVLDEAFQRYRDLLFGSGSWPRPYLTGKRHTLEKNVLVVSVVTPGCNQLPTLESVENYTLTIN
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
65  CVVLDEAFRRYRNLLFGSGSWPRPSFSNKQOTLGKNILVVSVVTAECNEFPNLESVENYTLTIN

128 DDQCLLLSETVWGALRGLTFSQLVWKS AEGTFF INKTEIEDFRPFPHRGLLLDTSRHYLPLSS
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
129 DDQCLLASETVWGALRGLTFSQLVWKS AEGTFF INKTKIKDFPRPFPHRGVLLDTSRHYLPLSS

192 ILDTLDVMAYNKLNVPFHWHLVDDPSFPYESFTFPELMRKGSYNPVTHIYTAQDVKEVIEYARLR
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
193 ILDTLDVMAYNKFNVPFHWHLVDDSSFPYESFTFPELTRKGSFNPVTHIYTAQDVKEVIEYARLR

256 GIRVLAEFDTPGHTLSWGP GIPGLLTPCYSGSEPSGTFGPVNP SLNNTYEFMSTFFLEVSSVFP
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
257 GIRVLAEFDTPGHTLSWGP GAPGLLTPCYSGSHLSGTFGPVNP SLNSTYDFMSTLFLEISSVFP

320 DFYLHLGGDEVDFTCWKS NPEIQDFMRKKGFGE DFKQLESFYIQTLLDIVSSYGKGYVWQEVF
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
321 DFYLHLGGDEVDFTCWKS NPIQAFMKKKGF-TDFKQLESFYIQTLLDIVSDYDKGYVWQEVF

384 DNKVKIQPDTIIQVWREDIPVNYMKELELVTKAGFRALLSAPWYLNRI SYGPDWKDFYVVEPLA
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
384 DNKVKVRPDTIIQVWREMPVEYMLEMQDITRAGFRALLSAPWYLN RVKYGPDWKDMYKVEPLA

448 FEGTPEQKALVIGGEACMWGEYVDNTNLVPRLWPRAGAVAERLWSNKLTS DLFAYERLSHFRCL
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
448 FHGTPEQKALVIGGEACMWGEYVDS TNLVPRLWPRAGAVAERL WSSNLT TNIDFAFKRLSHFRCL

512 ELLRRGVQAQPLNVGFCEQEFEQT*APGTEEGAGC
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
512 ELVRRGIQAQPI SVGCCEQEFEQT*ATSAEHPGGC
```

## Sequence Tool

### In this section...

“Overview of the Sequence Tool” on page 2-42

“Importing a Sequence” on page 2-42

“Viewing Nucleotide Sequence Information” on page 2-44

“Searching for Words” on page 2-46

“Exploring Open Reading Frames” on page 2-48

“Viewing Amino Acid Sequence Statistics” on page 2-51

“Closing the Sequence Tool” on page 2-55

“References” on page 2-55

### Overview of the Sequence Tool

The Sequence Tool window integrates many of the sequence functions in the toolbox. Instead of entering commands in the MATLAB Command Window, you can select and enter options.

### Importing a Sequence

The first step when analyzing a nucleotide or amino acid sequence is to import sequence information into the MATLAB environment. The Sequence Tool can connect to Web databases such as NCBI and EMBL and read information into the MATLAB environment.

The following procedure illustrates how to retrieve sequence information from the NCBI database on the Web. This example uses the GenBank accession number NM\_000520, which is the human gene HEXA that is associated with Tay-Sachs disease.

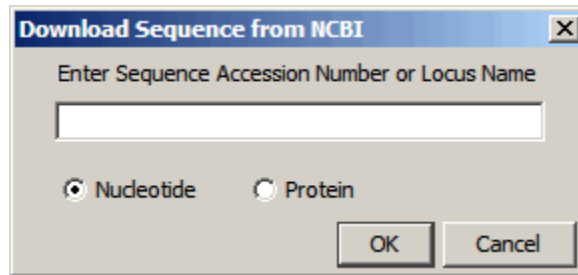
**1** In the MATLAB Command Window, type

```
seqtool
```

The Sequence Tool window opens without a sequence loaded. Notice that the panes to the right and bottom are blank.

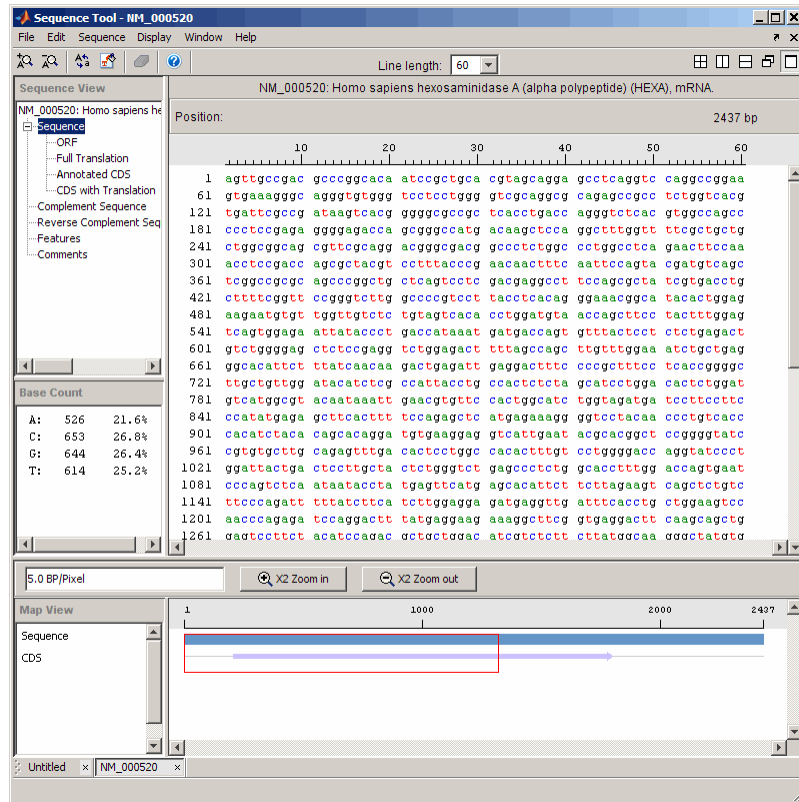
- 2 To retrieve a sequence from the NCBI database, select **File > Download Sequence from > NCBI**.

The Download Sequence from NCBI dialog box opens.



- 3 In the **Enter Sequence** box, type an accession number for an NCBI database entry, for example, **NM\_000520**. Click the **Nucleotide** option button, and then click **OK**.

The MATLAB software accesses the NCBI database on the Web, loads nucleotide sequence information for the accession number you entered, and calculates some basic statistics.



## Viewing Nucleotide Sequence Information

After you import a sequence into the Sequence Tool window, you can read information stored with the sequence, or you can view graphic representations for ORFs and CDSs.

- 1 In the left pane tree, click **Comments**. The right pane displays general information about the sequence.
- 2 Now click **Features**. The right pane displays NCBI feature information, including index numbers for a gene and any CDS sequences.
- 3 Click **ORF** to show the search results for ORFs in the six reading frames.

The screenshot displays the Sequence Tool interface for the sequence NM\_000520: Homo sapiens hexosaminidase A (alpha polypeptide) (HEXA), mRNA. The main window shows the nucleotide sequence with a line length of 60 bp. The sequence is displayed in a color-coded format, with the annotated CDS highlighted in red. The sequence is as follows:

```
1 agttgcccag gccctggcaca atccgctgca cgttagcagga gccctcaggcc caggccggaa
61 gtgaaagggc aggggtgtggg tctctctggg gtcgcagggc cagagccgcc tctggtcacg
121 tgatttcgcc ataagtcacg ggggcccgc tcacctgacc agggctctcac gtggccagcc
181 ccctccgaga ggggagacaa gggggccatg acaagctcca ggctttggtt ttcgctgctg
241 ctggcggcag cgttcgcagg acggggcagc gccctctggc cctggcctca gaacttccaa
```

The Base Count table is as follows:

Base	Count	Percentage
A	526	21.6%
C	653	26.8%
G	644	26.4%
T	614	25.2%

The Map View at the bottom shows the annotated CDS as a red bar with arrows indicating the direction of the sequence. The ORF and CDS are also visible in the Map View.

- 4 Click **Annotated CDS** to show the protein coding part of a nucleotide sequence.

The screenshot displays the 'Sequence Tool - NM\_000520' application window. The main window shows the DNA sequence for 'NM\_000520: Homo sapiens hexosaminidase A (alpha polypeptide) (HEXA), mRNA'. The sequence is displayed in a grid format with positions 10, 20, 30, 40, 50, and 60 marked. The sequence is as follows:

```

1 agttgcccag gccgggcaca atccgtgca cgtagcagga gccccaggcc caggccggaa
61 gtgaaagggc aggggtggyg tctctctggg gtccgagggc cagagccgcc tctggtcacg
121 tgattccgcc ataagtcacg gggggccgcg tcacctgacc agggctccac gtggccagcc
181 cctcccagaga ggggagacta gccggccatg acaagctcca ggccttgggt ttcgtgctg

241 ctggcggcag cgttcgcagg acggggcagc gccctctggc cctggccctca gaacttccaa
301 acctccgacc agcgttacgt cctttaccg aacaacttcc aattecagta cgtatgcagc
361 ctggcgcgcg agcccgctg ctcagtcttc gacgagccct tccagcgeta tcgtgacctg
421 cttttcgggt ccgggtcttg gccccgtct tacctcacg ggaaacggca tacactggag
481 aagaatgtgt tgggtgtctc tgtagtcaaa cctggatgta accagcttcc taacttggag
541 tcagtgagaa attataacct gaccataaat gatgaccagt gtttactcct ctctgagact
601 gtctggggag ctctccgagg tctggagact tttagccagc ttgtttggaa atctgctgag
661 ggcacattct ttatcaacaa gactgagatt gaggattttc cccgctttcc taaccggggc
721 ttactattaa atacatctca ccattacct ccaactctca acatcttada cactctgdat
  
```

Below the sequence, a 'Base Count' table is shown:

Base	Count	Percentage
A:	526	21.6%
C:	653	26.8%
G:	644	26.4%
T:	614	25.2%

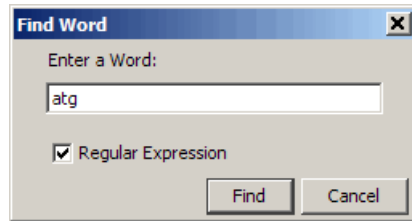
The 'Map View' at the bottom shows a graphical representation of the sequence with various features highlighted: ORFs (Open Reading Frames) in red and green, and CDS (Coding DNA Sequences) in blue. The map view includes a scale from 1 to 2437 bp and zoom controls.

## Searching for Words

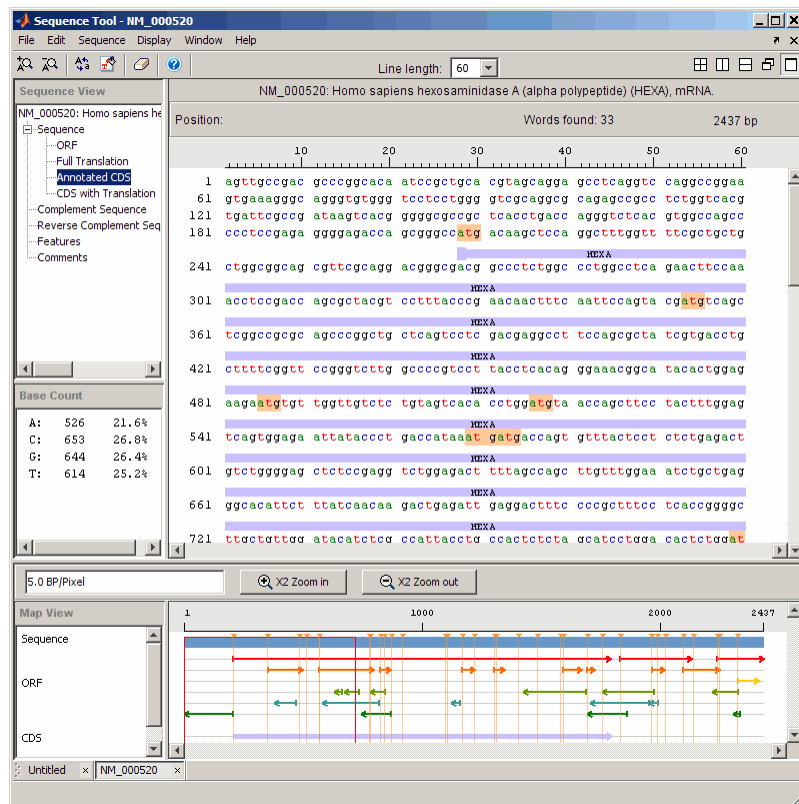
The following procedure illustrates how to search for characteristic words and sequence patterns. You will search for sequence patterns like the TATAA box and patterns for specific restriction enzymes.


- 1 Select **Sequence > Find Word**.
- 2 In the Find Word dialog box, type a sequence word or pattern, for example, **atg**, and then click **Find**.





The Sequence Tool window searches and displays the location of the selected word.



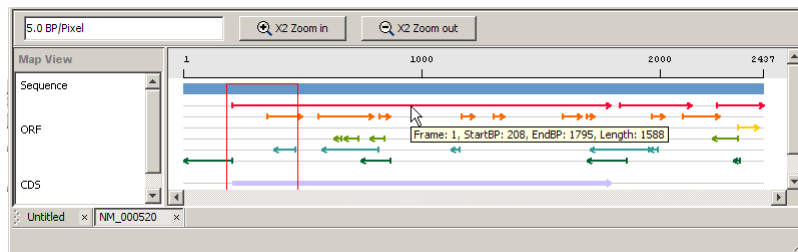
- 3 Clear the display by clicking the Clear Word Selection button  on the toolbar.

### Exploring Open Reading Frames

The following procedure illustrates how to identify the protein coding part of a nucleotide sequence and copy it into a new view. Identifying coding sections of a nucleotide sequence is a common bioinformatics task. After locating the coding part of a sequence, you can copy it to a new view, translate it to an amino acid sequence, and continue with your analysis.

- 1 In the left pane, click **ORF**.

The Sequence Tool window displays the ORFs for the six reading frames in the lower-right pane. Hover the cursor over a frame to display information about it.

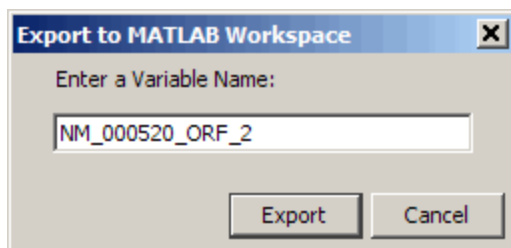


- 2 Click the longest ORF on reading frame 2.

The ORF is highlighted to indicate the part of the sequence that is selected.



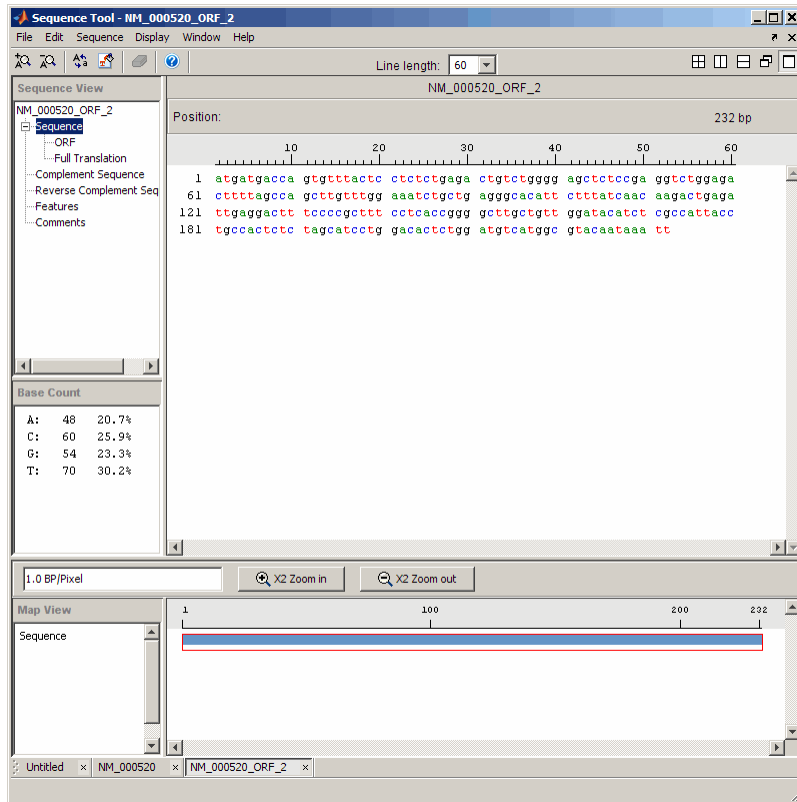
- 3 Right-click the selected ORF and then select **Export to Workspace**. In the Export to MATLAB Workspace dialog box, type a variable name, for example, `NM_000520_ORF_2`, then click **Export**.



The **NM\_000520\_ORF\_2** variable is added to the MATLAB Workspace.

- 4** Select **File > Import from Workspace**. Type the name of a variable with an exported ORF, for example, **NM\_000520\_ORF\_2**, and then click **Import**.

The Sequence Tool window adds a tab at the bottom for the new sequence while leaving the original sequence open.



- 5 In the left pane, click **Full Translation**. Select **Display > Amino Acid Residue Display > One Letter Code**.

The Sequence Tool window displays the amino acid sequence below the nucleotide sequence.

Sequence View

NM\_000520\_ORF\_2

- Sequence
  - ORF
    - Full Translation
  - Complement Sequence
  - Reverse Complement Seq
  - Features
  - Comments

Line length: 60

Position: 232 bp

1 atgatgacca ggtcttactc ctccttgaga ctgctctggg agctctccga ggtctggaga  
 M M T S V Y S S L R L S G E L S E V W R  
 \* P V F T P L \* D C L G S S P R S G D  
 D D Q C L L L S E T V W G A L R G L E  
 61 cttttagcca gctttttgg aaacttgctg agggcacatt ctttatcaac aagactgaga  
 L L A S L F G N L L R A H S L S T R L R  
 F P A C L E I C \* G H I L Y Q Q D \* D  
 T F S Q L V W K S A E G T F F I N K T E  
 121 ttgaggactt tccccgcttt cctcaccggg gcttgcctt ggaacatct cggcattacc  
 L R T F P A F L T G A C C W I H L A I T  
 \* G L S P L S S P G L A V G Y I S P L P  
 I E D F P R F P H R G L L L D T S R H Y  
 181 tggcaactctc tagcatcctg gacactctgg atgcatggc gtcaataaaa tt  
 C H S L A S W T L W H S W R T I N  
 A T L \* H P G H S G C H G V Q \* I  
 L P L S S I L D T L D V H A Y N K

Base Count

A:	48	20.7%
C:	60	25.9%
G:	54	23.3%
T:	70	30.2%

1.0 BP/Pixel X2 Zoom in X2 Zoom out

Map View

Sequence

1 100 200 232

Untitled x NM\_000520 x NM\_000520\_ORF\_2 x

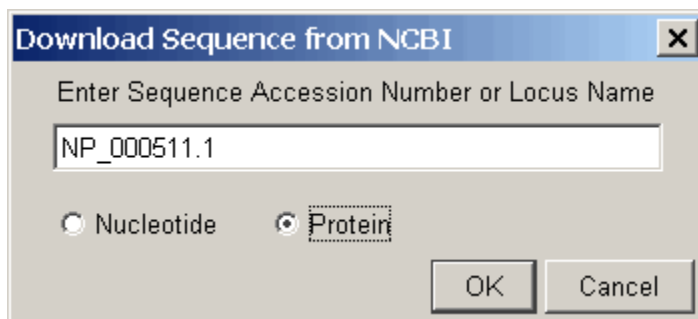
## Viewing Amino Acid Sequence Statistics

The following procedure illustrates how to view an amino acid sequence for an ORF located in a nucleotide sequence. You can import your own amino acid sequence, or you can get a protein sequence from the GenBank database. This example uses the GenBank accession number NP\_000511.1, which is the alpha subunit for a human enzyme associated with Tay-Sachs disease.

### 1 Select File > Download Sequence from > NCBI.

The Download Sequence from NCBI dialog box opens.

- 2 In the **Enter Sequence** box, type an accession number for an NCBI database entry, for example, **NP\_000511.1**. Click the **Protein** option button, and then click **OK**.



The MATLAB software accesses the NCBI database on the Web and loads amino acid sequence information for the accession number you entered.

Sequence Tool - NP\_000511

File Edit Sequence Display Window Help

Line length: 60

Sequence View

NP\_000511: hexosaminidase A preproprotein [Homo sapiens]

Position: 529 aa

```

1  mtssrlwfsl llaaafagra talwpwpqnf qtsdqryvly pnnfqfydv ssaapqpcsv
61  ldeafqyrd lffgsgwpv pytlgkhtl eknvlvsvv tpgcnlptl esvenyltli
121 nddqclllse tvwgalrgle tfsqlwkksa egtffinkte iedfprfphr gllldtsrhy
181 lpssildtl dvmaynklnv fhwhlvdps fpyesftfpe lmrkgsynpv thlytaqdvk
241 evieyarlrg irvlaefdtg ghtlswgpgi pglitpcysg sepstgfgpv npslnntyef
301 astfflevss vfpdfylhlg gdevdfcwk snpeiqlfmr kkgfgedfkq lesfyiqtlil
361 divssykggy vvwqevfdnk vkiqpdtiq vvreidipvny mkelelvtka gfrallsapw
421 ylnrisygpv wkdfyvvepl afegtpeqka lviggeacaw gezydntnlv prlwpzagav
481 acrlwnsklt sdltfayerl shfrcellrr gvqaqplnvq fceqefeqt

```

Amino Acid Count

A:	26	4.9%
R:	26	4.9%
N:	22	4.2%
D:	27	5.1%
C:	8	1.5%
Q:	22	4.2%
E:	36	6.8%

1.0 AA/Pixel X2 Zoom in X2 Zoom out

Map View

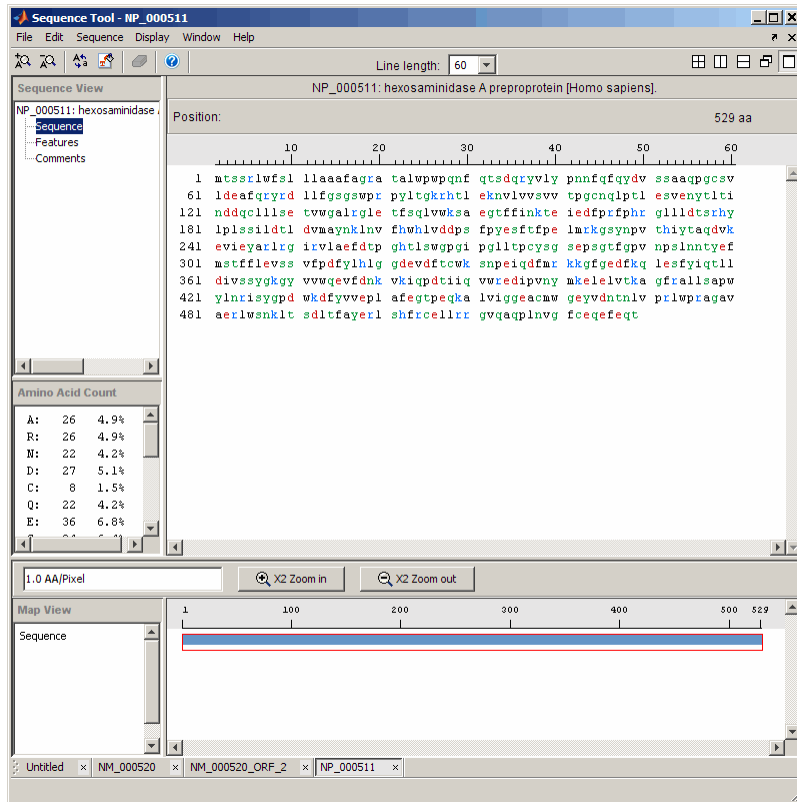
Sequence

1 100 200 300 400 500 529

Untitled x NM\_000520 x NM\_000520\_ORF\_2 x NP\_000511 x

- 3** Select **Display > Amino Acid Color Scheme**, and then select **Charge**, **Function**, **Hydrophobicity**, **Structure**, or **Taylor**. For example, select **Function**.

The display colors change to highlight charge information about the amino acid residues. The following table shows color legends for the amino acid color schemes.



Amino Acid Color Scheme	Color Legend
Charge	<ul style="list-style-type: none"> <li>• Acidic — Red</li> <li>• Basic — Light Blue</li> <li>• Neutral — Black</li> </ul>
Function	<ul style="list-style-type: none"> <li>• Acidic — Red</li> <li>• Basic — Light Blue</li> <li>• Hydrophobic, nonpolar — Black</li> <li>• Polar, uncharged — Green</li> </ul>



Amino Acid Color Scheme	Color Legend
Hydrophobicity	<ul style="list-style-type: none"><li>• Hydrophilic — Light Blue</li><li>• Hydrophobic — Black</li></ul>
Structure	<ul style="list-style-type: none"><li>• Ambivalent — Dark Green</li><li>• External — Light Blue</li><li>• Internal — Orange</li></ul>
Taylor	Each amino acid is assigned its own color, based on the colors proposed by W.R. Taylor.

## Closing the Sequence Tool

Close the Sequence Tool window from the MATLAB command line using the following syntax:

```
seqtool('close')
```

## References

[1] Taylor, W.R. (1997). Residual colours: a proposal for aminochromography. *Protein Engineering* 10, 7, 743–746.

## Multiple Sequence Alignment Viewer

In this section...
“Overview of the Multiple Sequence Alignment Viewer” on page 2-56
“Loading Sequence Data and Viewing the Phylogenetic Tree” on page 2-56
“Selecting a Subset of Data from the Phylogenetic Tree” on page 2-57
“Aligning Multiple Sequences” on page 2-58
“Adjusting Multiple Sequence Alignments Manually” on page 2-59
“Closing the Multiple Sequence Alignment Viewer” on page 2-62

### Overview of the Multiple Sequence Alignment Viewer

The Multiple Sequence Alignment Viewer is a graphical user interface (GUI) that integrates many sequence and multiple alignment functions in the toolbox. Instead of entering commands in the MATLAB Command Window, you can use this interface to visually inspect a multiple alignment and make manual adjustments.

### Loading Sequence Data and Viewing the Phylogenetic Tree

Load unaligned sequence data into the MATLAB environment and view it in a phylogenetic tree.

**1** Load sequence data.

```
load primatesdemodata
```

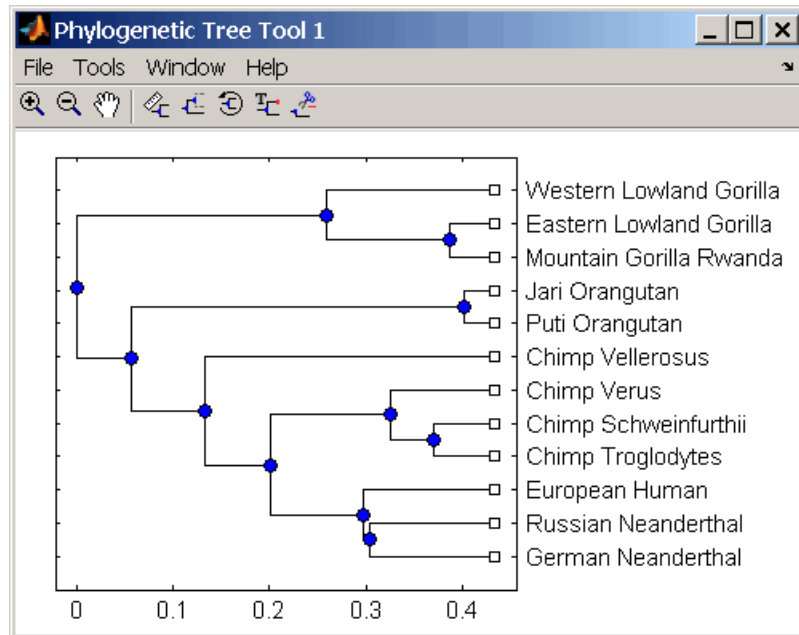
**2** Create a phylogenetic tree.

```
tree = seqlinkage(seqpdist(primates), 'single', primates);
```

**3** View the phylogenetic tree.

```
view(tree)
```

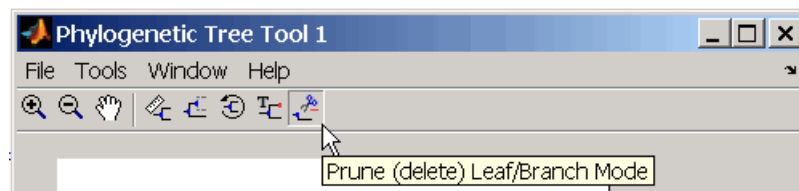
The MATLAB software creates a phytree object in the workspace and loads the sequence data into the Phylogenetic Tree Tool.



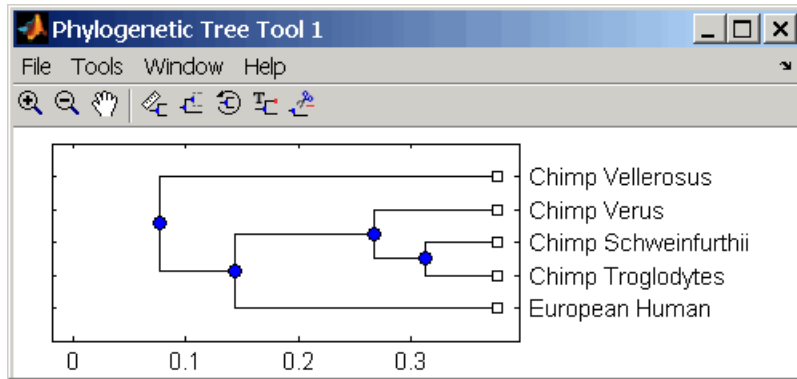
## Selecting a Subset of Data from the Phylogenetic Tree

Select the human and chimp branches.

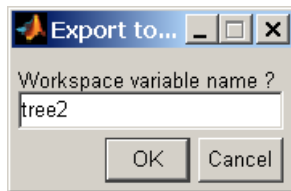
- 1 From the toolbar, click the **Prune** icon.



- 2 Click the branches to prune (remove) from the tree. For this example, click the branch nodes for gorillas, orangutans, and Neanderthals.



- 3 Export the selected branches to a second tree. Select **File > Export to Workspace**, and then select **Only Displayed**.
- 4 In the Export to dialog box, enter the name of a variable. For example, enter **tree2**, and then click **OK**.



- 5 Extract sequences from the tree object.

```
primates2 = primates(seqmatch(get(tree2, 'Leafnames'),{primates.Header}));
```

### Aligning Multiple Sequences

After selecting a set of related sequences, you can multiply align them and view the results.

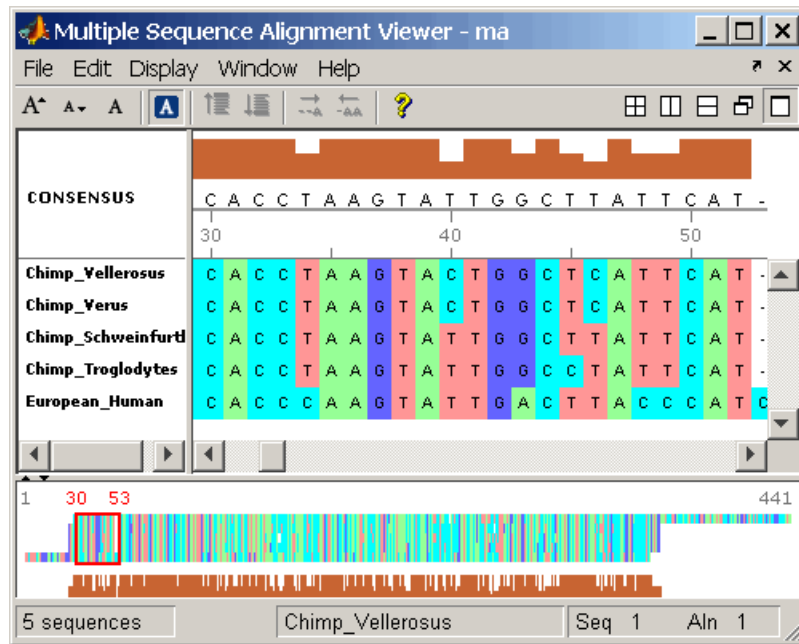
- 1 Align multiple sequences.

```
ma = multialign(primates2);
```

- 2 Load aligned sequences in the Multiple Sequence Alignment Viewer.

```
multialignviewer(ma);
```

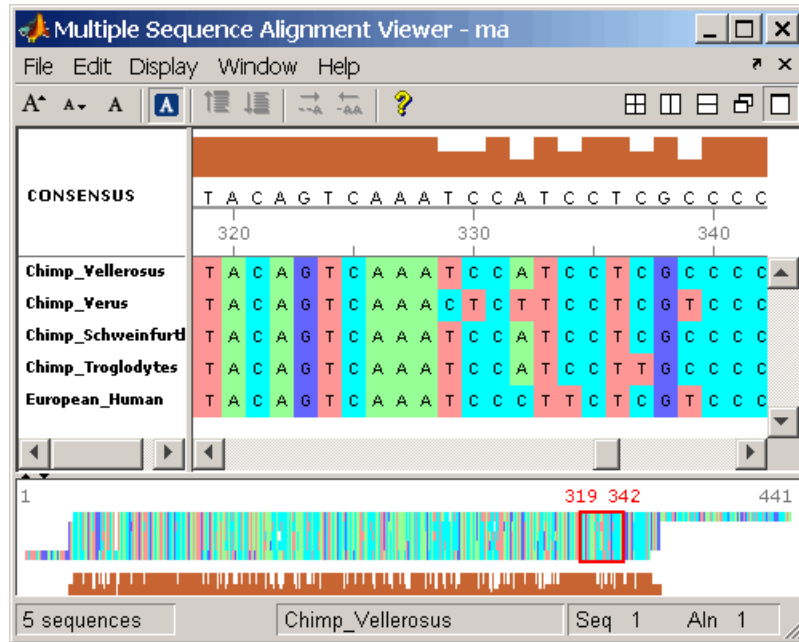
The aligned sequences appear in the Multiple Sequence Alignment Viewer.



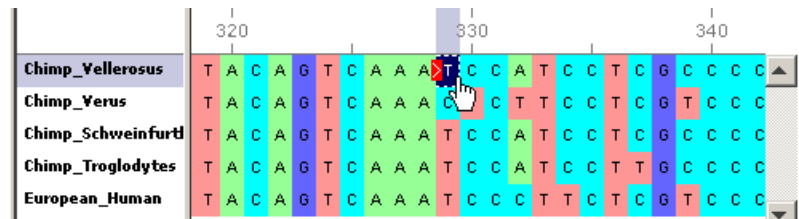
## Adjusting Multiple Sequence Alignments Manually

Algorithms for aligning multiple sequences do not always produce an optimal result. By visually inspecting the alignment, you can identify areas that could use a manual adjustment to improve the alignment.

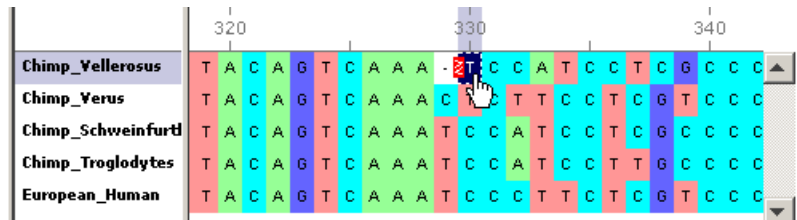
- 1 Identify an area where you could improve the alignment.



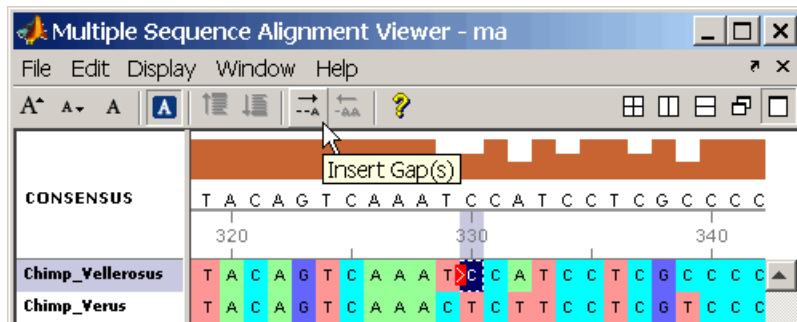
- 2 Click a letter to select it, and then move the cursor over the red direction bar. The cursor changes to a hand.



- 3 Click and drag the sequence to the right to insert a gap. If there is a gap to the left, you can also move the sequence to the left and eliminate the gap.

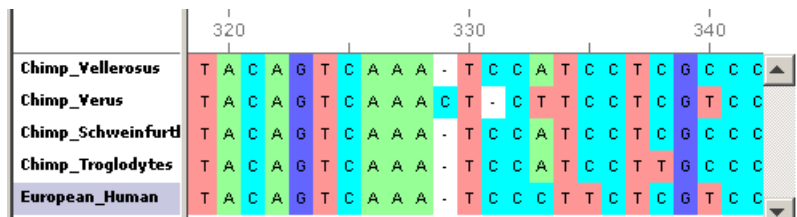


Alternately, to insert a gap, select a character, and then click the **Insert Gap** icon on the toolbar or press the spacebar.



**Note** You cannot delete or add letters to a sequence, but you can add or delete gaps. If all of the sequences at one alignment position have gaps, you can delete that column of gaps.

#### 4 Continue adding gaps and moving sequences to improve the alignment.



### **Closing the Multiple Sequence Alignment Viewer**

Close the Multiple Sequence Alignment Viewer window from the MATLAB command line using the following syntax:

```
multialignviewer('close')
```



## Storing and Managing Short-Read Sequence Data in Objects

### In this section...

“Overview” on page 2-63

“Representing Sequence and Quality Data in a BioRead Object” on page 2-64

“Representing Sequence, Quality, and Alignment/Mapping Data in a BioMap Object” on page 2-66

“Retrieving Information from a BioRead or BioMap Object” on page 2-70

“Setting Information in a BioRead or BioMap Object” on page 2-72

“Determining Coverage of a Reference Sequence” on page 2-73

“Constructing Sequence Alignments to a Reference Sequence” on page 2-75

“Filtering Read Sequences Using SAM Flags” on page 2-76

### Overview

High-throughput sequencing instruments produce large amounts of short-read sequence data that can be a challenge to store and manage. Using objects to contain this data lets you easily access, manipulate, and filter the data.

Bioinformatics Toolbox includes two objects for working with short-read sequence data.

Object	Contains This Information	Construct from One of These
BioRead	<ul style="list-style-type: none"> <li>Sequence headers</li> <li>Read sequences</li> <li>Sequence qualities</li> </ul>	<ul style="list-style-type: none"> <li>BioIndexedFile object</li> <li>FASTQ file</li> <li>FASTQ structure (created using the <code>fastqread</code> function)</li> <li>Cell arrays containing header, sequence, and quality information</li> </ul>

Object	Contains This Information	Construct from One of These
		(created using the fastqread function) <ul style="list-style-type: none"> <li>• SAM file</li> </ul>
BioMap	<ul style="list-style-type: none"> <li>• Sequence headers</li> <li>• Read sequences</li> <li>• Sequence qualities</li> <li>• Sequence alignment and mapping information (relative to one reference sequence)</li> </ul>	<ul style="list-style-type: none"> <li>• BioIndexedFile object</li> <li>• SAM file</li> <li>• SAM structure (created using the samread function)</li> <li>• BAM structure (created using the bamread function)</li> <li>• Cell arrays containing header, sequence, quality, and mapping/alignment information (created using the samread or bamread function)</li> </ul>

## Representing Sequence and Quality Data in a BioRead Object

A BioRead object represents a collection of read sequences. Each element in the object is associated with a sequence, sequence header, and sequence quality information.

### Constructing a BioRead Object from a FASTQ-Formatted File

Use the BioRead constructor function to construct a BioRead object from a FASTQ-formatted file and set the Name property:

```
BRObj1 = BioRead('fastqfile', 'SRR005164_1_50.fastq', 'Name', 'MyObject')

BRObj1 =

    BioRead
```

```
Properties:
  Quality: {50x1 cell}
  Sequence: {50x1 cell}
  Header: {50x1 cell}
  NSeqs: 50
  Name: 'MyObject'
```

Methods, Superclasses

## Constructing a BioRead Object from a Very Large FASTQ-Formatted File

Some FASTQ-formatted files are very large and can cause an out-of-memory error when you use them as input to the `BioRead` function. If this happens, create a `BioIndexedFile` object, which lets you access the contents of the FASTQ-formatted file (up to 4 GB), without loading the file into memory. You can then use the `BioRead` constructor function to construct a `BioRead` object from the `BioIndexedFile` object.

- 1 Create a variable containing the full absolute path of your source FASTQ file. For your source file, use the `SRR005164_1_50.fastq` file, which is included with the Bioinformatics Toolbox software.

```
sourcefile = which('SRR005164_1_50.fastq');
```

- 2 Use the `BioIndexedFile` constructor function to construct a `BioIndexedFile` object from the `SRR005164_1_50.fastq` source file. Save the index file in the Current Folder.

```
FASTQBIFObj = BioIndexedFile('fastq', sourcefile, '.');
```

The `BioIndexedFile` constructor function constructs `FASTQBIFObj`, a `BioIndexedFile` object, and also creates an index file with the same name as the source file, but with an `IDX` extension. It stores this index file in the Current Folder because you specified this location.

---

**Caution** Do not modify the index file. If you modify it, you can get invalid results. Also, the constructor function cannot use a modified index file to create future objects from the associated source file.

---

- 3** Use the `BioRead` constructor function to construct a `BioRead` object from the `BioIndexedFile` object.

```
BRObj2 = BioRead(FASTQBIFObj)
```

```
BRObj2 =
```

```
  BioRead
```

```
  Properties:
```

```
    Quality: [50x1 File indexed property]
```

```
    Sequence: [50x1 File indexed property]
```

```
    Header: [50x1 File indexed property]
```

```
    NSeqs: 50
```

```
    Name: ''
```

```
  Methods, Superclasses
```

---

**Note** Because you constructed this `BioRead` object from a `BioIndexedFile` object, you cannot modify the properties (except for the `Name` property) of the `BioRead` object.

---

## Representing Sequence, Quality, and Alignment/Mapping Data in a `BioMap` Object

A `BioMap` object represents a collection of read sequences that map against a single reference sequence. Each element in the object is associated with a read sequence, sequence header, sequence quality information, and alignment/mapping information.

## Constructing a BioMap Object from a SAM Structure

- 1 Use the `samread` function to create a SAM structure from a SAM-formatted file:

```
SAMStruct = samread('ex2.sam');
```

- 2 To construct a valid `BioMap` object from a SAM-formatted file, the file must contain only one reference sequence. Determine the number and names of the reference sequences in your SAM-formatted file using the `unique` function to find unique names in the `ReferenceName` field of the structure:

```
unique({SAMStruct.ReferenceName})
```

```
ans =
```

```
    'seq1'    'seq2'
```

- 3 Use the `BioMap` constructor function to construct a `BioMap` object from a SAM structure. Because the SAM structure contains multiple reference sequences, use the `SubsetRef` name-value pair argument to specify one reference sequence, `seq1`:

```
BMObj1 = BioMap(SAMStruct, 'SubsetRef', 'seq1')
```

```
BMObj1 =
```

```
    BioMap
```

```
Properties:
```

```
    Reference: 'seq1'
    Signature: {1501x1 cell}
    Start: [1501x1 uint32]
MappingQuality: [1501x1 uint8]
    Flag: [1501x1 uint16]
    MatePosition: [1501x1 uint32]
    Quality: {1501x1 cell}
    Sequence: {1501x1 cell}
    Header: {1501x1 cell}
    NSeqs: 1501
    Name: ''
```

Methods, Superclasses

### **Constructing a BioMap Object from a SAM-Formatted File**

Use the `BioMap` constructor function to construct a `BioMap` object from a SAM-formatted file and set the `Name` property. Because the SAM-formatted file in this example, `ex2.sam`, contains multiple reference sequences, use the `SubsetRef` name-value pair argument to specify one reference sequence, `seq1`:

```
BMObj2 = BioMap('samfile', 'ex2.sam', 'SubsetRef', 'seq1', 'Name', 'MyObject')
```

```
BMObj2 =
```

```
  BioMap
```

```
Properties:
```

```
    Reference: 'seq1'  
    Signature: {1501x1 cell}  
      Start: [1501x1 uint32]  
MappingQuality: [1501x1 uint8]  
      Flag: [1501x1 uint16]  
    MatePosition: [1501x1 uint32]  
      Quality: {1501x1 cell}  
    Sequence: {1501x1 cell}  
      Header: {1501x1 cell}  
      NSeqs: 1501  
      Name: 'MyObject'
```

Methods, Superclasses

### **Constructing a BioMap Object from a Very Large SAM-Formatted File**

Some SAM-formatted files are very large and can cause an out-of-memory error when you use them as input to the `BioMap` function. If this happens, create a `BioIndexedFile` object to access the contents of the SAM-formatted file (up to 4 GB), without loading the file into memory. Then use the `BioMap` constructor function to construct a `BioMap` object from the `BioIndexedFile` object.

- 1 Create a variable containing the full absolute path of your source SAM file. For your source file, use the `ex2.sam` file, which is included with the Bioinformatics Toolbox software.

```
sourcefile = which('ex2.sam');
```

- 2 Use the `BioIndexedFile` constructor function to construct a `BioIndexedFile` object from the `ex2.sam` source file. Save the index file in the Current Folder.

```
SAMBIFObj = BioIndexedFile('sam', sourcefile, '.');
```

The `BioIndexedFile` constructor function constructs `SAMBIFObj`, a `BioIndexedFile` object, and also creates an index file with the same name as the source file, but with an `IDX` extension. It stores this index file in the Current Folder as specified.

---

**Caution** Do not modify the index file, otherwise you can get invalid results. Also, the constructor function cannot use a modified index file to create future objects from the associated source file.

---

- 3 Use the `getDictionary` method to determine the number and names of the reference sequences in the SAM-formatted file associated with the `SAMBIFObj`, the `BioIndexedFile` object:

```
getDictionary(SAMBIFObj)
```

```
ans =
```

```
    'seq1'
```

```
    'seq2'
```

- 4 Use the `BioMap` constructor function to construct a `BioMap` object from the `BioIndexedFile` object. Because the SAM-formatted file contains multiple reference sequences, use the `SubsetRef` name-value pair argument to specify one reference sequence, `seq1`:

```
BMObj3 = BioMap(SAMBIFObj, 'SubsetRef', 'seq1')
```

```
BMObj3 =
```

BioMap

Properties:

```
Reference: 'seq1'  
Signature: [1501x1 File indexed property]  
Start: [1501x1 File indexed property]  
MappingQuality: [1501x1 File indexed property]  
Flag: [1501x1 File indexed property]  
MatePosition: [1501x1 File indexed property]  
Quality: [1501x1 File indexed property]  
Sequence: [1501x1 File indexed property]  
Header: [1501x1 File indexed property]  
NSeqs: 1501  
Name: ''
```

Methods, Superclasses

---

**Note** Because you constructed this BioMap object from a BioIndexedFile object, you cannot modify the properties (except for Name and Reference) of the BioMap object.

---

## Retrieving Information from a BioRead or BioMap Object

You can retrieve all or a subset of information from a BioRead or BioMap object.

### Retrieving All Values of a Property from a BioRead or BioMap Object

Use the `get` method to retrieve a specific property from all elements in a BioRead or BioMap object. For example, to retrieve all headers from a BioRead object, use the `get` method with the `Header` property:

```
allHeaders = get(BRObj1, 'Header');
```

The previous syntax returns a cell array containing the headers for all the elements in the BioRead object. For example, to retrieve all start positions of



aligned read sequences from a `BioMap` object, use the `get` method with the `Start` property:

```
allStarts = get(BMObj1, 'Start');
```

The previous syntax returns a vector containing the start positions of aligned read sequences with respect to the position numbers in the reference sequence in a `BioMap` object.

---

**Note** Property names are case sensitive. For a list and description of all properties of a `BioRead` object, see `BioRead` class. For a list and description of all properties of a `BioMap` object, see `BioMap` class.

---

## Retrieving a Subset of Information from a `BioRead` or `BioMap` Object

Use specialized `get` methods with a numeric vector, logical vector, or cell array of headers to retrieve a subset of information from an object. For example, to retrieve the first 10 elements from a `BioRead` object, use the `getSubset` method:

```
newBRObj = getSubset(BRObj1, [1:10]);
```

The previous syntax returns a new `BioRead` object containing the first 10 elements in the original `BioRead` object.

For example, to retrieve the first 12 positions of sequences with headers `SRR005164.1`, `SRR005164.7`, and `SRR005164.16`, use the `getSubsequence` method:

```
subSeqs = getSubsequence(BRObj1, ...  
                        {'SRR005164.1', 'SRR005164.7', 'SRR005164.16'}, [1:12]')
```

```
subSeqs =  
  
    'TGGCTTTAAAGC'  
    'CCCGAAAGCTAG'  
    'AATTTTGCGGCT'
```

For example, to retrieve information about the third element in a `BioMap` object, use the `getInfo` method:

```
Info_3 = getInfo(BMObj1, 3);
```

The previous syntax returns a tab-delimited string containing this information for the third element:

- Sequence header
- SAM flags for the sequence
- Start position of the aligned read sequence with respect to the reference sequence
- Mapping quality score for the sequence
- Signature (CIGAR-formatted string) for the sequence
- Sequence
- Quality scores for sequence positions

---

**Note** Method names are case sensitive. For a complete list and description of methods of a `BioRead` object, see `BioRead` class. For a complete list and description of methods of a `BioMap` object, see `BioMap` class.

---

### Setting Information in a `BioRead` or `BioMap` Object

Several specialized set methods let you set the properties of a subset of elements in a `BioRead` or `BioMap` object.

---

**Note** If you construct a `BioRead` or `BioMap` object from a `BioIndexedFile` object, you cannot modify the properties of the object (except for `Name` and `Reference`).

---

### Providing Custom Headers for Sequences

To provide custom headers for sequences of interest (in this case sequences 2, 4, and 6), use the `setHeader` method:

```
newBRObj = setHeader(BRObj1, {'H2', 'H4', 'H6'}, [2 4 6]);
```

The previous syntax returns a new object containing the new headers.

### Renaming the Reference Sequence

To rename the reference sequence in a `BioMap` object, use the `setReference` method:

```
BMObj1 = setReference(BMObj1, 'Chromosome7');
```

The previous syntax updates the name of the reference sequence from `seq1` to `Chromosome7` in the `BioMap` object.

---

**Note** Method names are case sensitive. For a complete list and description of methods of a `BioRead` object, see `BioRead` class. For a complete list and description of methods of a `BioMap` object, see `BioMap` class.

---

### Determining Coverage of a Reference Sequence

When working with a `BioMap` object, you can determine the number of read sequences that:

- Align within a specific region of the reference sequence
- Align to each position within a specific region of the reference sequence

For example, you can compute the number, indices, and start positions of the read sequences that align within the first 25 positions of the reference sequence. To do so, use the `getCounts`, `getIndex`, and `getStart` methods:

```
Cov = getCounts(BMObj1, 1, 25)
```

```
Cov =
```

```
12
```

```
Indices = getIndex(BMObj1, 1, 25)
```

```
Indices =
```

```
1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12
```

```
startPos = getStart(BMObj1, Indices)
```

```
startPos =
```

```
1  
3  
5  
6  
9  
13  
13  
15  
18  
22  
22  
24
```

The first two syntaxes return the number and indices of the read sequences that align within the specified region of the reference sequence. The last syntax returns a vector containing the start position of each aligned read sequence, corresponding to the position numbers of the reference sequence.

For example, you can also compute the number of the read sequences that align to *each* of the first 10 positions of the reference sequence. For this computation, use the `getBaseCoverage` method:

```
Cov = getBaseCoverage(BMObj1, 1, 10)
```

```
Cov =
```

```
    1    1    2    2    3    4    4    4    5    5
```

## Constructing Sequence Alignments to a Reference Sequence

It is useful to construct and view the alignment of the read sequences that align to a specific region of the reference sequence. It is also helpful to know which read sequences align to this region in a BioMap object.

For example, to retrieve the alignment of read sequences to the first 12 positions of the reference sequence in a BioMap object, use the `getAlignment` method:

```
[Alignment_1_12, Indices] = getAlignment(BMObj2, 1, 12)
```

```
Alignment_1_12 =
```

```
CACTAGTGGCTC
CTAGTGGCTC
AGTGGCTC
GTGGCTC
GCTC
```

```
Indices =
```

```
1
2
3
4
5
```

Return the headers of the read sequences that align to a specific region of the reference sequence:

```
alignedHeaders = getHeader(BMObj2, Indices)
```

```
alignedHeaders =  
  
  'B7_591:4:96:693:509'  
  'EAS54_65:7:152:368:113'  
  'EAS51_64:8:5:734:57'  
  'B7_591:1:289:587:906'  
  'EAS56_59:8:38:671:758'
```

## Filtering Read Sequences Using SAM Flags

SAM-formatted files include the status of 11 flags for each read sequence. These flags describe different sequencing and alignment aspects of a read sequence. For more information on the flags, see the SAM format specification. The `filterByFlag` method lets you filter the read sequences in a `BioMap` object by using these flags.

## Removing Unmapped Read Sequences

- 1 Create a `BioMap` object from a SAM-formatted file.

```
BMObj2 = BioMap('samfile', 'ex1.sam');
```

- 2 Use the `filterByFlag` method to create a logical vector indicating the read sequences in a `BioMap` object that are mapped.

```
LogicalVec_mapped = filterByFlag(BMObj2, 'unmappedQuery', false);
```

- 3 Use this logical vector and the `getSubset` method to create a new `BioMap` object containing only the mapped read sequences.

```
filteredBMObj_1 = getSubset(BMObj2, LogicalVec_mapped);
```

## Removing Read Sequences That Are Not Mapped in a Pair

- 1 Create a `BioMap` object from a SAM-formatted file.

```
BMObj2 = BioMap('samfile', 'ex1.sam');
```

- 2 Use the `filterByFlag` method to create a logical vector indicating the read sequences in a `BioMap` object that are mapped in a proper pair, that is, both the read sequence and its mate are mapped to the reference sequence.

```
LogicalVec_paired = filterByFlag(BMObj2, 'pairedInMap', true);
```

- 3** Use this logical vector and the `getSubset` method to create a new `BioMap` object containing only the read sequences that are mapped in a proper pair.

```
filteredBMObj_2 = getSubset(BMObj2, LogicalVec_paired);
```





# Microarray Analysis

---

- “Storing and Managing Gene Expression Data in Objects” on page 3-2
- “Representing Expression Data Values in DataMatrix Objects” on page 3-5
- “Representing Expression Data Values in ExptData Objects” on page 3-11
- “Representing Sample and Feature Metadata in MetaData Objects” on page 3-15
- “Representing Experiment Information in a MIAME Object” on page 3-22
- “Representing All Data and Information in an ExpressionSet Object” on page 3-27
- “Example: Visualizing Microarray Data” on page 3-33
- “Example: Analyzing Gene Expression Profiles” on page 3-57

## Storing and Managing Gene Expression Data in Objects

Microarray gene expression experiments are complex, containing data and information from various sources. The data and information from such an experiment is typically subdivided into four categories:

- Measured expression data values
- Sample metadata
- Microarray feature metadata
- Descriptions of experiment methods and conditions

In MATLAB, you can represent all the previous data and information in an `ExpressionSet` object, which typically contains the following objects:

- One `ExprData` object containing expression values from a microarray experiment in one or more `DataMatrix` objects
- One `MetaData` object containing *sample* metadata in two dataset arrays
- One `MetaData` object containing *feature* metadata in two dataset arrays
- One `MIAME` object containing experiment descriptions

The following graphic illustrates a typical `ExpressionSet` object and its component objects.

## ExpressionSet object

## ExptData object

DataMatrix object

DataMatrix object

DataMatrix object

## MetaData object (sample information)

dataset array

dataset array

## MetaData object (feature information)

dataset array

dataset array

## MIAME object

Each element (DataMatrix object) in the ExpressionSet object has an element name. Also, there is always one DataMatrix object whose element name is Expressions.

An ExpressionSet object lets you store, manage, and subset the data from a microarray gene expression experiment. An ExpressionSet object includes properties and methods that let you access, retrieve, and change data, metadata, and other information about the microarray experiment. These properties and methods are useful to view and analyze the data. For a list of the properties and methods, see ExpressionSet class.

To learn more about constructing and using objects for microarray gene expression data and information, see:

- “Representing Expression Data Values in DataMatrix Objects” on page 3-5
- “Representing Expression Data Values in ExptData Objects” on page 3-11
- “Representing Sample and Feature Metadata in MetaData Objects” on page 3-15
- “Representing Experiment Information in a MIAME Object” on page 3-22
- “Representing All Data and Information in an ExpressionSet Object” on page 3-27

# Representing Expression Data Values in DataMatrix Objects

## In this section...

“Overview of DataMatrix Objects” on page 3-5

“Constructing DataMatrix Objects” on page 3-6

“Getting and Setting Properties of a DataMatrix Object” on page 3-7

“Accessing Data in DataMatrix Objects” on page 3-8

## Overview of DataMatrix Objects

The toolbox includes functions, objects, and methods for creating, storing, and accessing microarray data.

The object constructor function, `DataMatrix`, lets you create a `DataMatrix` object to encapsulate data and metadata (row and column names) from a microarray experiment. A `DataMatrix` object stores experimental data in a matrix, with rows typically corresponding to gene names or probe identifiers, and columns typically corresponding to sample identifiers. A `DataMatrix` object also stores metadata, including the gene names or probe identifiers (as the row names) and sample identifiers (as the column names).

You can reference microarray expression values in a `DataMatrix` object the same way you reference data in a MATLAB array, that is, by using linear or logical indexing. Alternately, you can reference this experimental data by gene (probe) identifiers and sample identifiers. Indexing by these identifiers lets you quickly and conveniently access subsets of the data without having to maintain additional index arrays.

Many MATLAB operators and arithmetic functions are available to `DataMatrix` objects by means of methods. These methods let you modify, combine, compare, analyze, plot, and access information from `DataMatrix` objects. Additionally, you can easily extend the functionality by using general element-wise functions, `dmarrayfun` and `dmbsxfun`, and by manually accessing the properties of a `DataMatrix` object.

---

**Note** For tables describing the properties and methods of a `DataMatrix` object, see the `DataMatrix` object reference page.

---

## Constructing `DataMatrix` Objects

- 1 Load the MAT-file, provided with the Bioinformatics Toolbox software, that contains yeast data. This MAT-file includes three variables: `yeastvalues`, a 614-by-7 matrix of gene expression data, `genes`, a cell array of 614 GenBank accession numbers for labeling the rows in `yeastvalues`, and `times`, a 1-by-7 vector of time values for labeling the columns in `yeastvalues`.

```
load filteredyeastdata
```

- 2 Create variables to contain a subset of the data, specifically the first five rows and first four columns of the `yeastvalues` matrix, the `genes` cell array, and the `times` vector.

```
yeastvalues = yeastvalues(1:5,1:4);  
genes = genes(1:5,:);  
times = times(1:4);
```

- 3 Import the microarray object package so that the `DataMatrix` constructor function will be available.

```
import bioma.data.*
```

- 4 Use the `DataMatrix` constructor function to create a small `DataMatrix` object from the gene expression data in the variables you created in step 2.

```
dmo = DataMatrix(yeastvalues,genes,times)
```

```
dmo =
```

	0	9.5	11.5	13.5
SS DNA	-0.131	1.699	-0.026	0.365
YAL003W	0.305	0.146	-0.129	-0.444
YAL012W	0.157	0.175	0.467	-0.379
YAL026C	0.246	0.796	0.384	0.981

```
YAL034C    -0.235    0.487    -0.184    -0.669
```

## Getting and Setting Properties of a DataMatrix Object

You use the `get` and `set` methods to retrieve and set properties of a `DataMatrix` object.

- 1 Use the `get` method to display the properties of the `DataMatrix` object, `dmo`.

```
get(dmo)
      Name: ''
      RowNames: {5x1 cell}
      ColNames: {' 0' ' 9.5' '11.5' '13.5'}
      NRows: 5
      NCols: 4
      NDims: 2
      ElementClass: 'double'
```

- 2 Use the `set` method to specify a name for the `DataMatrix` object, `dmo`.

```
dmo = set(dmo, 'Name', 'MyDMObject');
```

- 3 Use the `get` method again to display the properties of the `DataMatrix` object, `dmo`.

```
get(dmo)
      Name: 'MyDMObject'
      RowNames: {5x1 cell}
      ColNames: {' 0' ' 9.5' '11.5' '13.5'}
      NRows: 5
      NCols: 4
      NDims: 2
      ElementClass: 'double'
```

---

**Note** For a description of all properties of a `DataMatrix` object, see the `DataMatrix` object reference page.

---

## Accessing Data in DataMatrix Objects

DataMatrix objects support the following types of indexing to extract, assign, and delete data:

- Parenthesis ( ) indexing
- Dot . indexing

### Parentheses ( ) Indexing

Use parenthesis indexing to extract a subset of the data in `dmo` and assign it to a new DataMatrix object `dmo2`:

```
dmo2 = dmo(1:5,2:3)
dmo2 =
           9.5    11.5
SS DNA    1.699   -0.026
YAL003W   0.146   -0.129
YAL012W   0.175    0.467
YAL026C   0.796    0.384
YAL034C   0.487   -0.184
```

Use parenthesis indexing to extract a subset of the data using row names and column names, and assign it to a new DataMatrix object `dmo3`:

```
dmo3 = dmo({'SS DNA', 'YAL012W', 'YAL034C'}, '11.5')
dmo3 =
           11.5
SS DNA    -0.026
YAL012W    0.467
YAL034C   -0.184
```

---

**Note** If you use a cell array of row names or column names to index into a DataMatrix object, the names must be unique, even though the row names or column names within the DataMatrix object are not unique.

---



Use parenthesis indexing to assign new data to a subset of the elements in `dmo2`:

```
dmo2({'SS DNA', 'YAL003W'}, 1:2) = [1.700 -0.030; 0.150 -0.130]
dmo2 =
```

	9.5	11.5
SS DNA	1.7	-0.03
YAL003W	0.15	-0.13
YAL012W	0.175	0.467
YAL026C	0.796	0.384
YAL034C	0.487	-0.184

Use parenthesis indexing to delete a subset of the data in `dmo2`:

```
dmo2({'SS DNA', 'YAL003W'}, :) = []
dmo2 =
```

	9.5	11.5
YAL012W	0.175	0.467
YAL026C	0.796	0.384
YAL034C	0.487	-0.184

## Dot . Indexing

---

**Note** In the following examples, notice that when using dot indexing with DataMatrix objects, you specify all rows or all columns using a colon within single quotation marks, (':').

---

Use dot indexing to extract the data from the 11.5 column only of `dmo`:

```
timeValues = dmo.(':')('11.5')
timeValues =
```

```
-0.0260
-0.1290
 0.4670
 0.3840
-0.1840
```

Use dot indexing to assign new data to a subset of the elements in `dmo`:

```
dmo.(1:2)(':') = 7  
dmo =
```

	0	9.5	11.5	13.5
SS DNA	7	7	7	7
YAL003W	7	7	7	7
YAL012W	0.157	0.175	0.467	-0.379
YAL026C	0.246	0.796	0.384	0.981
YAL034C	-0.235	0.487	-0.184	-0.669

Use dot indexing to delete an entire variable from `dmo`:

```
dmo.YAL034C = []  
dmo =
```

	0	9.5	11.5	13.5
SS DNA	7	7	7	7
YAL003W	7	7	7	7
YAL012W	0.157	0.175	0.467	-0.379
YAL026C	0.246	0.796	0.384	0.981

Use dot indexing to delete two columns from `dmo`:

```
dmo.(':')(2:3)=[ ]  
dmo =
```

	0	13.5
SS DNA	7	7
YAL003W	7	7
YAL012W	0.157	-0.379
YAL026C	0.246	0.981

## Representing Expression Data Values in ExptData Objects

### In this section...

“Overview of ExptData Objects” on page 3-11

“Constructing ExptData Objects” on page 3-12

“Using Properties of an ExptData Object” on page 3-12

“Using Methods of an ExptData Object” on page 3-13

“References” on page 3-14

### Overview of ExptData Objects

You can use an ExptData object to store expression values from a microarray experiment. An ExptData object stores the data values in one or more DataMatrix objects, each having the same row names (feature names) and column names (sample names). Each element (DataMatrix object) in the ExptData object has an element name.

The following illustrates a small DataMatrix object containing expression values from three samples (columns) and seven features (rows):

	A	B	C
100001_at	2.26	20.14	31.66
100002_at	158.86	236.25	206.27
100003_at	68.11	105.45	82.92
100004_at	74.32	96.68	84.87
100005_at	75.05	53.17	57.94
100006_at	80.36	42.89	77.21
100007_at	216.64	191.32	219.48

An ExptData object lets you store, manage, and subset the data values from a microarray experiment. An ExptData object includes properties and methods that let you access, retrieve, and change data values from a microarray experiment. These properties and methods are useful to view and analyze the data. For a list of the properties and methods, see ExptData class.

## Constructing ExptData Objects

The mouseExprsData.txt file used in this example contains data from Hovatta et al., 2005.

- 1 Import the bioma.data package so that the DataMatrix and ExptData constructor functions are available.

```
import bioma.data.*
```

- 2 Use the DataMatrix constructor function to create a DataMatrix object from the gene expression data in the mouseExprsData.txt file. This file contains a table of expression values and metadata (sample and feature names) from a microarray experiment done using the Affymetrix MGU74Av2 GeneChip array. There are 26 sample names (A through Z), and 500 feature names (probe set names).

```
dmObj = DataMatrix('File', 'mouseExprsData.txt');
```

- 3 Use the ExptData constructor function to create an ExptData object from the DataMatrix object.

```
EDObj = ExptData(dmObj);
```

- 4 Display information about the ExptData object, EDObj.

```
EDObj
```

```
Experiment Data:  
500 features, 26 samples  
1 elements  
Element names: Elmt1
```

---

**Note** For complete information on constructing ExptData objects, see ExptData class.

---

## Using Properties of an ExptData Object

To access properties of an ExptData object, use the following syntax:

```
objectname.propertyname
```

For example, to determine the number of elements (DataMatrix objects) in an ExptData object:

```
EDObj.NElements  
  
ans =  
  
1
```

To set properties of an ExptData object, use the following syntax:

```
objectname.propertyname = propertyvalue
```

For example, to set the Name property of an ExptData object:

```
EDObj.Name = 'MyExptDataObject'
```

---

**Note** Property names are case sensitive. For a list and description of all properties of an ExptData object, see ExptData class.

---

## Using Methods of an ExptData Object

To use methods of an ExptData object, use either of the following syntaxes:

```
objectname.methodname
```

or

```
methodname(objectname)
```

For example, to retrieve the sample names from an ExptData object:

```
EDObj.sampleNames  
  
Columns 1 through 9  
  
'A'    'B'    'C'    'D'    'E'    'F'    'G'    'H'    'I'    ...
```

To return the size of an ExptData object:

```
size(EDObj)
```

```
ans =  
500 26
```

---

**Note** For a complete list of methods of an `ExptData` object, see `ExptData` class.

---

## References

[1] Hovatta, I., Tennant, R S., Helton, R., et al. (2005). Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. *Nature* 438, 662–666.

## Representing Sample and Feature Metadata in MetaData Objects

### In this section...

“Overview of MetaData Objects” on page 3-15

“Constructing MetaData Objects” on page 3-16

“Using Properties of a MetaData Object” on page 3-19

“Using Methods of a MetaData Object” on page 3-20

### Overview of MetaData Objects

You can store either sample or feature metadata from a microarray gene expression experiment in a MetaData object. The metadata consists of variable names, for example, related to either samples or microarray features, along with descriptions and values for the variables.

A MetaData object stores the metadata in two dataset arrays:

- **Values dataset array** — A dataset array containing the measured value of each variable per sample or feature. In this dataset array, the columns correspond to variables and rows correspond to either samples or features. The number and names of the columns in this dataset array must match the number and names of the rows in the Descriptions dataset array. If this dataset array contains *sample* metadata, then the number and names of the rows (samples) must match the number and names of the columns in the DataMatrix objects in the same ExpressionSet object. If this dataset array contains *feature* metadata, then the number and names of the rows (features) must match the number and names of the rows in the DataMatrix objects in the same ExpressionSet object.
- **Descriptions dataset array** — A dataset array containing a list of the variable names and their descriptions. In this dataset array, each row corresponds to a variable. The row names are the variable names, and a column, named `VariableDescription`, contains a description of the variable. The number and names of the rows in the Descriptions dataset array must match the number and names of the columns in the Values dataset array.

The following illustrates a dataset array containing the measured value of each variable per sample or feature:

	Gender	Age	Type	Strain	Source
A	'Male'	8	'Wild type'	'129S6/SvEvTac'	'amygdala'
B	'Male'	8	'Wild type'	'129S6/SvEvTac'	'amygdala'
C	'Male'	8	'Wild type'	'129S6/SvEvTac'	'amygdala'
D	'Male'	8	'Wild type'	'A/J '	'amygdala'
E	'Male'	8	'Wild type'	'A/J '	'amygdala'
F	'Male'	8	'Wild type'	'C57BL/6J '	'amygdala'

The following illustrates a dataset array containing a list of the variable names and their descriptions:

	VariableDescription
id	'Sample identifier'
Gender	'Gender of the mouse in study'
Age	'The number of weeks since mouse birth'
Type	'Genetic characters'
Strain	'The mouse strain'
Source	'The tissue source for RNA collection'

A `MetaData` object lets you store, manage, and subset the metadata from a microarray experiment. A `MetaData` object includes properties and methods that let you access, retrieve, and change metadata from a microarray experiment. These properties and methods are useful to view and analyze the metadata. For a list of the properties and methods, see `MetaData` class

## Constructing MetaData Objects

### Constructing a MetaData Object from Two dataset Arrays

- 1 Import the `bioma.data` package so that the `MetaData` constructor function is available.

```
import bioma.data.*
```

- 2 Load some sample data, which includes Fisher's iris data of 5 measurements on a sample of 150 irises.



```
load fisheriris
```

- 3** Create a dataset array from some of Fisher's iris data. The dataset array will contain 750 measured values, one for each of 150 samples (iris replicates) at five variables (species, SL, SW, PL, PW). In this dataset array, the rows correspond to samples, and the columns correspond to variables.

```
irisValues = dataset({nominal(species), 'species'}, ...
                    {meas, 'SL', 'SW', 'PL', 'PW'});
```

- 4** Create another dataset array containing a list of the variable names and their descriptions. This dataset array will contain five rows, each corresponding to the five variables: species, SL, SW, PL, and PW. The first column will contain the variable name. The second column will have a column header of VariableDescription and contain a description of the variable.

```
% Create 5-by-1 cell array of description text for the variables
varDesc = {'Iris species', 'Sepal Length', 'Sepal Width', ...
           'Petal Length', 'Petal Width'};
% Create the dataset array from the variable descriptions
irisVarDesc = dataset(varDesc, ...
                      'ObsNames', {'species', 'SL', 'SW', 'PL', 'PW'}, ...
                      'VarNames', {'VariableDescription'})
```

```
irisVarDesc =
```

	VariableDescription
species	'Iris species'
SL	'Sepal Length'
SW	'Sepal Width'
PL	'Petal Length'
PW	'Petal Width'

- 5** Create a MetaData object from the two dataset arrays.

```
MDObj1 = MetaData(irisValues, irisVarDesc);
```

## Constructing a MetaData Object from a Text File

- 1 Import the `bioma.datapackage` so that the `MetaData` constructor function is available.

```
import bioma.data.*
```

- 2 View the `mouseSampleData.txt` file included with the Bioinformatics Toolbox software.

Note that this text file contains two tables. One table contains 130 measured values, one for each of 26 samples (A through Z) at five variables (Gender, Age, Type, Strain, and Source). In this table, the rows correspond to samples, and the columns correspond to variables. The second table has lines prefaced by the `#` symbol. It contains five rows, each corresponding to the five variables: Gender, Age, Type, Strain, and Source. The first column contains the variable name. The second column has a column header of `VariableDescription` and contains a description of the variable.

```
# id: Sample identifier
# Gender: Gender of the mouse in study
# Age: The number of weeks since mouse birth
# Type: Genetic characters
# Strain: The mouse strain
# Source: The tissue source for RNA collection
ID Gender Age Type Strain Source
A Male 8 Wild type 129S6/SvEvTac amygdala
B Male 8 Wild type 129S6/SvEvTac amygdala
C Male 8 Wild type 129S6/SvEvTac amygdala
D Male 8 Wild type A/J amygdala
E Male 8 Wild type A/J amygdala
F Male 8 Wild type C57BL/6J amygdala
G Male 8 Wild type C57BL/6J amygdala
H Male 8 Wild type 129S6/SvEvTac cingulate cortex
I Male 8 Wild type 129S6/SvEvTac cingulate cortex
J Male 8 Wild type A/J cingulate cortex
K Male 8 Wild type A/J cingulate cortex
L Male 8 Wild type A/J cingulate cortex
M Male 8 Wild type C57BL/6J cingulate cortex
N Male 8 Wild type C57BL/6J cingulate cortex
```

```

O Male 8 Wild type 129S6/SvEvTac hippocampus
P Male 8 Wild type 129S6/SvEvTac hippocampus
Q Male 8 Wild type A/J hippocampus
R Male 8 Wild type A/J hippocampus
S Male 8 Wild type C57BL/6J hippocampus
T Male 8 Wild type C57BL/6J4 hippocampus
U Male 8 Wild type 129S6/SvEvTac hypothalamus
V Male 8 Wild type 129S6/SvEvTac hypothalamus
W Male 8 Wild type A/J hypothalamus
X Male 8 Wild type A/J hypothalamus
Y Male 8 Wild type C57BL/6J hypothalamus
Z Male 8 Wild type C57BL/6J hypothalamus

```

- 3** Create a MetaData object from the metadata in the mouseSampleData.txt file.

```
MDObj2 = MetaData('File', 'mouseSampleData.txt', 'VarDescChar', '#')
```

Sample Names:

A, B, ...,Z (26 total)

Variable Names and Meta Information:

	VariableDescription
Gender	' Gender of the mouse in study'
Age	' The number of weeks since mouse birth'
Type	' Genetic characters'
Strain	' The mouse strain'
Source	' The tissue source for RNA collection'

For complete information on constructing MetaData objects, see MetaData class.

## Using Properties of a MetaData Object

To access properties of a MetaData object, use the following syntax:

```
objectname.propertyname
```

For example, to determine the number of variables in a MetaData object:

```
MDObj2.NVariables
```

```
ans =
```

```
5
```

To set properties of a `MetaData` object, use the following syntax:

```
objectname.propertyname = propertyvalue
```

For example, to set the `Description` property of a `MetaData` object:

```
MDObj1.Description = 'This is my MetaData object for my sample metadata'
```

---

**Note** Property names are case sensitive. For a list and description of all properties of a `MetaData` object, see `MetaData` class.

---

## Using Methods of a MetaData Object

To use methods of a `MetaData` object, use either of the following syntaxes:

```
objectname.methodname
```

or

```
methodname(objectname)
```

For example, to access the dataset array in a `MetaData` object that contains the variable values:

```
MDObj2.variableValues;
```

To access the dataset array of a `MetaData` object that contains the variable descriptions:

```
variableDesc(MDObj2)
```

```
ans =
```

```
                VariableDescription
Gender          ' Gender of the mouse in study'
Age             ' The number of weeks since mouse birth'
```

```
Type      ' Genetic characters'  
Strain    ' The mouse strain'  
Source    ' The tissue source for RNA collection'
```

---

**Note** For a complete list of methods of a MetaData object, see MetaData class.

---

## Representing Experiment Information in a MIAME Object

In this section...
“Overview of MIAME Objects” on page 3-22
“Constructing MIAME Objects” on page 3-22
“Using Properties of a MIAME Object” on page 3-25
“Using Methods of a MIAME Object” on page 3-25

### Overview of MIAME Objects

You can store information about experimental methods and conditions from a microarray gene expression experiment in a MIAME object. It loosely follows the Minimum Information About a Microarray Experiment (MIAME) specification. It can include information about:

- Experiment design
- Microarrays used
- Samples used
- Sample preparation and labeling
- Hybridization procedures and parameters
- Normalization controls
- Preprocessing information
- Data processing specifications

A MIAME object includes properties and methods that let you access, retrieve, and change experiment information related to a microarray experiment. These properties and methods are useful to view and analyze the information. For a list of the properties and methods, see MIAME class.

### Constructing MIAME Objects

For complete information on constructing MIAME objects, see MIAME class.

## Constructing a MIAME Object from a GEO Structure

- 1 Import the `bioma.data` package so that the MIAME constructor function is available.

```
import bioma.data.*
```

- 2 Use the `getgeodata` function to return a MATLAB structure containing Gene Expression Omnibus (GEO) Series data related to accession number GSE4616.

```
geoStruct = getgeodata('GSE4616')
```

```
geoStruct =
```

```
Header: [1x1 struct]
```

```
Data: [12488x12 bioma.data.DataMatrix]
```

- 3 Use the MIAME constructor function to create a MIAME object from the structure.

```
MIAMEObj1 = MIAME(geoStruct);
```

- 4 Display information about the MIAME object, `MIAMEObj1`.

```
MIAMEObj1
```

```
MIAMEObj1 =
```

```
Experiment Description:
```

```
Author name: Mika,,Silvennoinen
```

```
Riikka,,Kivel^/
```

```
Maarit,,Lehti
```

```
Anna-Maria,,Touvras
```

```
Jyrki,,Komulainen
```

```
Veikko,,Vihko
```

```
Heikki,,Kainulainen
```

```
Laboratory: LIKES - Research Center
```

```
Contact information: Mika,,Silvennoinen
```

```
URL:
```

```
PubMedIDs: 17003243
```

```
Abstract: A 90 word abstract is available. Use the Abstract property.  
Experiment Design: A 234 word summary is available. Use the ExptDesign property.  
Other notes:  
  [1x80 char]
```

## Constructing a MIAME Object from Properties

- 1 Import the `bioma.data` package so that the MIAME constructor function is available.

```
import bioma.data.*
```

- 2 Use the MIAME constructor function to create a MIAME object using individual properties.

```
MIAMEObj2 = MIAME('investigator', 'Jane Researcher',...  
                  'lab', 'One Bioinformatics Laboratory',...  
                  'contact', 'jresearcher@lab.not.exist',...  
                  'url', 'www.lab.not.exist',...  
                  'title', 'Normal vs. Diseased Experiment',...  
                  'abstract', 'Example of using expression data',...  
                  'other', {'Notes:Created from a text file.'});
```

- 3 Display information about the MIAME object, `MIAMEObj2`.

```
MIAMEObj2  
  
MIAMEObj2 =  
  
Experiment Description:  
Author name: Jane Researcher  
Laboratory: One Bioinformatics Laboratory  
Contact information: jresearcher@lab.not.exist  
URL: www.lab.not.exist  
PubMedIDs:  
Abstract: A 4 word abstract is available. Use the Abstract property.  
No experiment design summary available.  
Other notes:  
  'Notes:Created from a text file.'
```



## Using Properties of a MIAME Object

To access properties of a MIAME object, use the following syntax:

```
objectname.propertyname
```

For example, to retrieve the PubMed identifier of publications related to a MIAME object:

```
MIAMEObj1.PubMedID
```

```
ans =
```

```
17003243
```

To set properties of a MIAME object, use the following syntax:

```
objectname.propertyname = propertyvalue
```

For example, to set the Laboratory property of a MIAME object:

```
MIAMEObj1.Laboratory = 'XYZ Lab'
```

---

**Note** Property names are case sensitive. For a list and description of all properties of a MIAME object, see MIAME class.

---

## Using Methods of a MIAME Object

To use methods of a MIAME object, use either of the following syntaxes:

```
objectname.methodname
```

or

```
methodname(objectname)
```

For example, to determine if a MIAME object is empty:

```
MIAMEObj1.isempty
```

```
ans =
```

0

---

**Note** For a complete list of methods of a MIAME object, see MIAME class.

---

# Representing All Data and Information in an ExpressionSet Object

## In this section...

“Overview of ExpressionSet Objects” on page 3-27

“Constructing ExpressionSet Objects” on page 3-29

“Using Properties of an ExpressionSet Object” on page 3-30

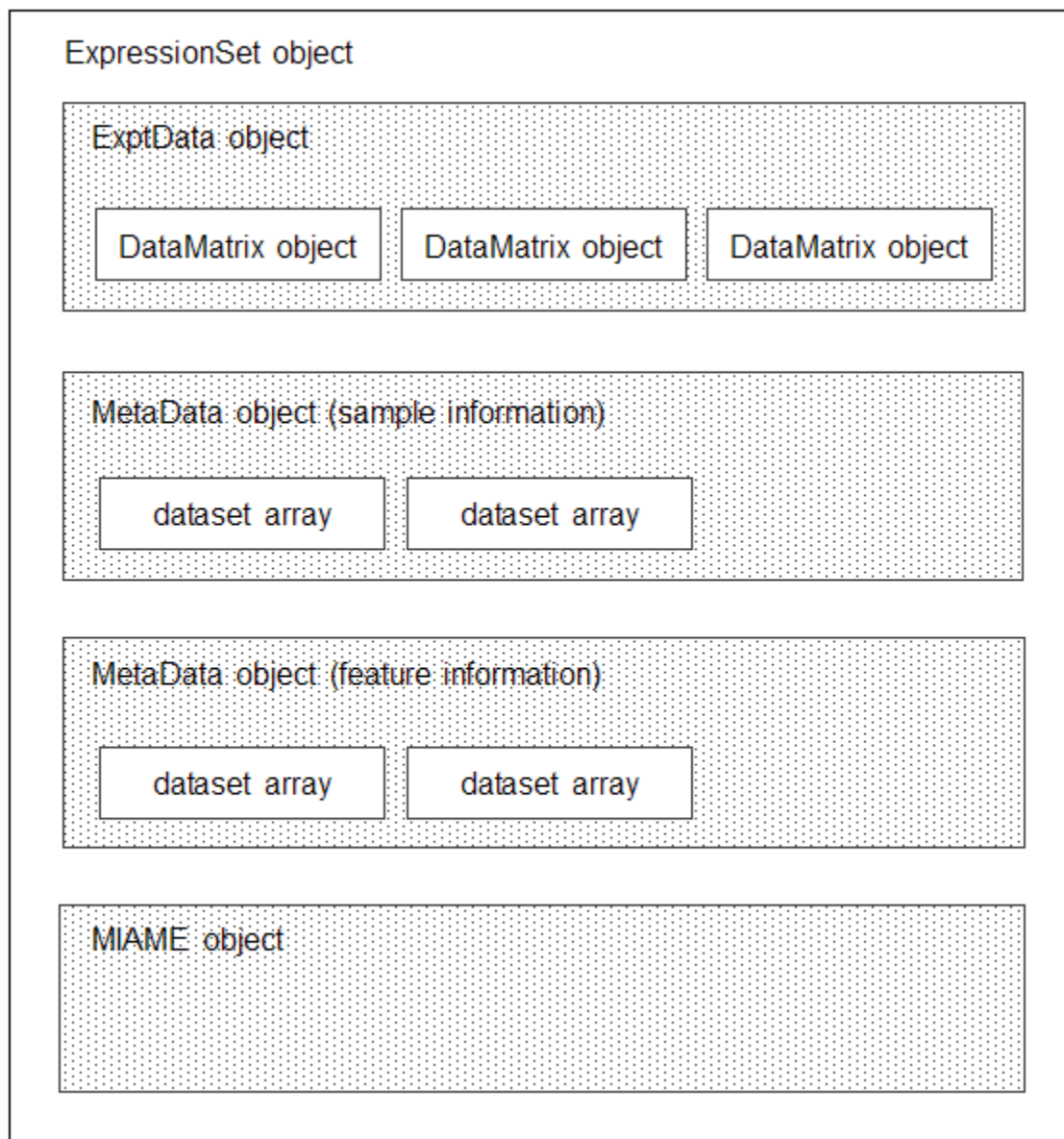
“Using Methods of an ExpressionSet Object” on page 3-30

## Overview of ExpressionSet Objects

You can store all microarray experiment data and information in one object by assembling the following into an ExpressionSet object:

- One ExptData object containing expression values from a microarray experiment in one or more DataMatrix objects
- One MetaData object containing *sample* metadata in two dataset arrays
- One MetaData object containing *feature* metadata in two dataset arrays
- One MIAME object containing experiment descriptions

The following graphic illustrates a typical ExpressionSet object and its component objects.



Each element (DataMatrix object) in the ExpressionSet object has an element name. Also, there is always one DataMatrix object whose element name is Expressions.

An ExpressionSet object lets you store, manage, and subset the data from a microarray gene expression experiment. An ExpressionSet object includes properties and methods that let you access, retrieve, and change data, metadata, and other information about the microarray experiment. These properties and methods are useful to view and analyze the data. For a list of the properties and methods, see ExpressionSet class.

## Constructing ExpressionSet Objects

---

**Note** The following procedure assumes you have executed the example code in the previous sections:

- “Representing Expression Data Values in ExptData Objects” on page 3-11
  - “Representing Sample and Feature Metadata in MetaData Objects” on page 3-15
  - “Representing Experiment Information in a MIAME Object” on page 3-22
- 

**1** Import the bioma package so that the ExpressionSet constructor function is available.

```
import bioma.*
```

**2** Construct an ExpressionSet object from EDObj, an ExptData object, MDObj2, a MetaData object containing sample variable information, and MIAMEObj, a MIAME object.

```
ESObj = ExpressionSet(EDObj, 'SData', MDObj2, 'EInfo', MIAMEObj1);
```

**3** Display information about the ExpressionSet object, ESObj.

```
ESObj
```

```
ExpressionSet
Experiment Data: 500 features, 26 samples
  Element names: Expressions
Sample Data:
  Sample names:      A, B, ...,Z (26 total)
  Sample variable names and meta information:
    Gender: Gender of the mouse in study
    Age: The number of weeks since mouse birth
    Type: Genetic characters
    Strain: The mouse strain
    Source: The tissue source for RNA collection
Feature Data: none
Experiment Information: use 'exptInfo(obj)'
```

For complete information on constructing ExpressionSet objects, see ExpressionSet class.

## Using Properties of an ExpressionSet Object

To access properties of an ExpressionSet object, use the following syntax:

```
objectname.propertyname
```

For example, to determine the number of samples in an ExpressionSet object:

```
ESObj.NSamples
ans =
    26
```

---

**Note** Property names are case sensitive. For a list and description of all properties of an ExpressionSet object, see ExpressionSet class.

---

## Using Methods of an ExpressionSet Object

To use methods of an ExpressionSet object, use either of the following syntaxes:

```
objectname.methodname
```

or

```
methodname(objectname)
```

For example, to retrieve the sample variable names from an ExpressionSet object:

```
ESObj.sampleVarNames
```

```
ans =
```

```
'Gender' 'Age' 'Type' 'Strain' 'Source'
```

To retrieve the experiment information contained in an ExpressionSet object:

```
exptInfo(ESObj)
```

```
ans =
```

```
Experiment description
```

```
  Author name: Mika,,Silvennoinen
```

```
Riikka,,Kivel^/
```

```
Maarit,,Lehti
```

```
Anna-Maria,,Touvras
```

```
Jyrki,,Komulainen
```

```
Veikko,,Vihko
```

```
Heikki,,Kainulainen
```

```
  Laboratory: XYZ Lab
```

```
  Contact information: Mika,,Silvennoinen
```

```
  URL:
```

```
  PubMedIDs: 17003243
```

```
  Abstract: A 90 word abstract is available Use the Abstract property.
```

```
  Experiment Design: A 234 word summary is available Use the ExptDesign property.
```

```
  Other notes:
```

```
    [1x80 char]
```

---

**Note** For a complete list of methods of an ExpressionSet object, see ExpressionSet class.

---



## Example: Visualizing Microarray Data

### In this section...

“Overview of the Mouse Example” on page 3-33

“Exploring the Microarray Data Set” on page 3-34

“Spatial Images of Microarray Data” on page 3-36

“Statistics of the Microarrays” on page 3-46

“Scatter Plots of Microarray Data” on page 3-48

### Overview of the Mouse Example

This example looks at the various ways to visualize microarray data. The data comes from a pharmacological model of Parkinson’s disease (PD) using a mouse brain. The microarray data for this example is from Brown, V.M., Ossadtchi, A., Khan, A.H., Yee, S., Lacan, G., Melega, W.P., Cherry, S.R., Leahy, R.M., and Smith, D.J.; "Multiplex three dimensional brain gene expression mapping in a mouse model of Parkinson’s disease"; *Genome Research* 12(6): 868-884 (2002).

The microarray data used in this example is available in a Web supplement to the paper by Brown et al. and in the file `mouse_a1pd.gpr` included with the Bioinformatics Toolbox software.

[http://labs.pharmacology.ucla.edu/smithlab/genome\\_multiplex/](http://labs.pharmacology.ucla.edu/smithlab/genome_multiplex/)

The microarray data is also available on the Gene Expression Omnibus Web site at

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE30>

The GenePix GPR-formatted file `mouse_a1pd.gpr` contains the data for one of the microarrays used in the study. This is data from voxel A1 of the brain of a mouse in which a pharmacological model of Parkinson’s disease (PD) was induced using methamphetamine. The voxel sample was labeled with Cy3 (green) and the control, RNA from a total (not voxelated) normal mouse brain, was labeled with Cy5 (red). GPR formatted files provide a large amount of information about the array, including the mean, median, and standard

deviation of the foreground and background intensities of each spot at the 635 nm wavelength (the red, Cy5 channel) and the 532 nm wavelength (the green, Cy3 channel).

## Exploring the Microarray Data Set

This procedure illustrates how to import data from the Web into the MATLAB environment, using data from a study about gene expression in mouse brains as an example. See “Overview of the Mouse Example” on page 3-33.

- 1 Read data from a file into a MATLAB structure. For example, in the MATLAB Command Window, type

```
pd = gprread('mouse_a1pd.gpr')
```

Information about the structure displays in the MATLAB Command Window:

```
pd =  
    Header: [1x1 struct]  
    Data: [9504x38 double]  
    Blocks: [9504x1 double]  
    Columns: [9504x1 double]  
    Rows: [9504x1 double]  
    Names: {9504x1 cell}  
    IDs: {9504x1 cell}  
    ColumnNames: {38x1 cell}  
    Indices: [132x72 double]  
    Shape: [1x1 struct]
```

- 2 Access the fields of a structure using `StructureName.FieldName`. For example, you can access the field `ColumnNames` of the structure `pd` by typing

```
pd.ColumnNames
```

The column names are shown below.

```
ans =  
    'X'  
    'Y'  
    'Dia.'
```

```

'F635 Median'
'F635 Mean'
'F635 SD'
'B635 Median'
'B635 Mean'
'B635 SD'
'% > B635+1SD'
'% > B635+2SD'
'F635 % Sat.'
'F532 Median'
'F532 Mean'
'F532 SD'
'B532 Median'
'B532 Mean'
'B532 SD'
'% > B532+1SD'
'% > B532+2SD'
'F532 % Sat.'
'Ratio of Medians'
'Ratio of Means'
'Median of Ratios'
'Mean of Ratios'
'Ratios SD'
'Rgn Ratio'
'Rgn Rt'
'F Pixels'
'B Pixels'
'Sum of Medians'
'Sum of Means'
'Log Ratio'
'F635 Median - B635'
'F532 Median - B532'
'F635 Mean - B635'
'F532 Mean - B532'
'Flags'

```

- 3** Access the names of the genes. For example, to list the first 20 gene names, type

```
pd.Names(1:20)
```

A list of the first 20 gene names is displayed:

```
ans =  
    'AA467053 '  
    'AA388323 '  
    'AA387625 '  
    'AA474342 '  
    'Myo1b '  
    'AA473123 '  
    'AA387579 '  
    'AA387314 '  
    'AA467571 '  
    ''  
    'Spop '  
    'AA547022 '  
    'AI508784 '  
    'AA413555 '  
    'AA414733 '  
    ''  
    'Snta1 '  
    'AI414419 '  
    'W14393 '  
    'W10596 '
```

## Spatial Images of Microarray Data

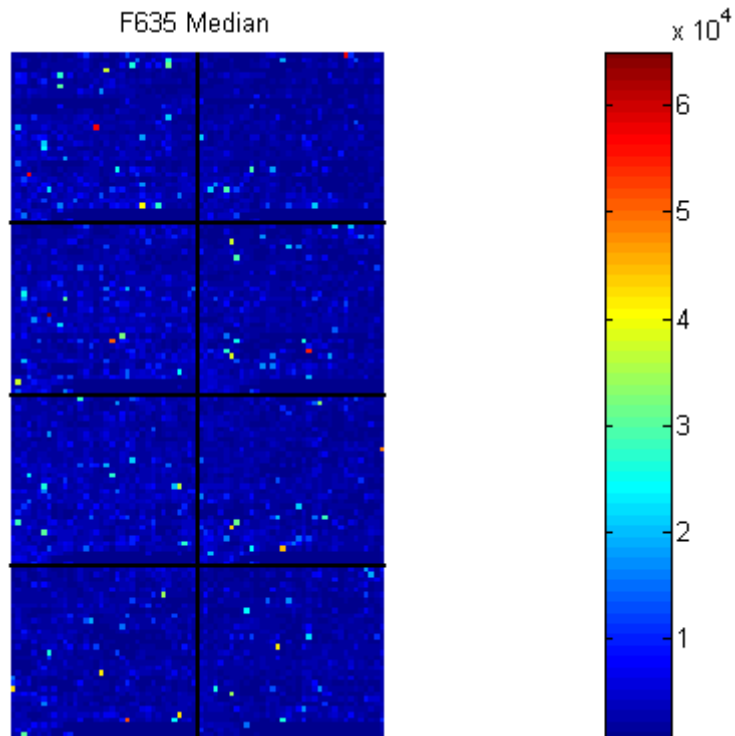
This procedure illustrates how to visualize microarray data by plotting image maps. The function `mimage` can take a microarray data structure and create a pseudocolor image of the data arranged in the same order as the spots on the array. In other words, `mimage` plots a spatial plot of the microarray.

This procedure uses data from a study of gene expression in mouse brains. For a list of field names in the MATLAB structure `pd`, see “Exploring the Microarray Data Set” on page 3-34.

- 1 Plot the median values for the red channel. For example, to plot data from the field `F635 Median`, type

```
figure  
mimage(pd, 'F635 Median')
```

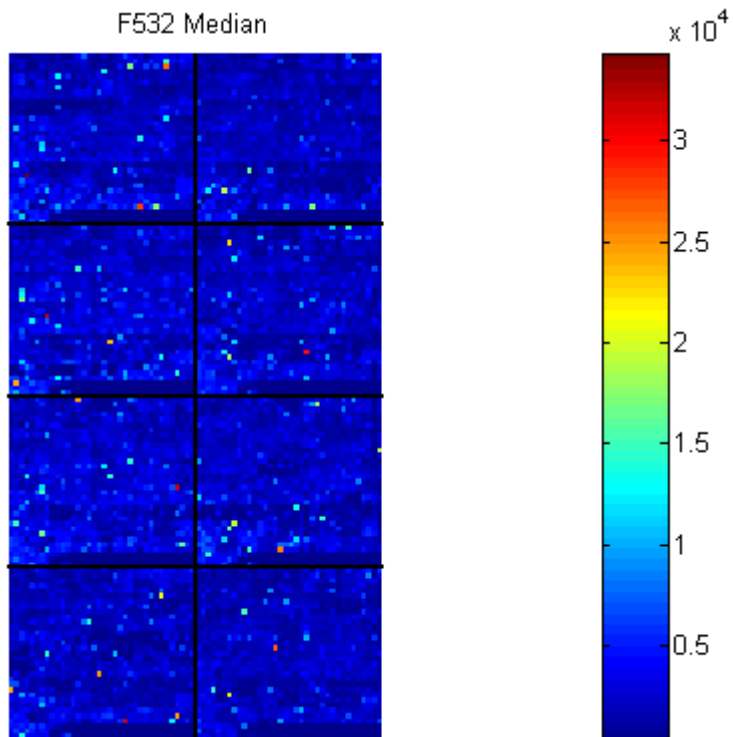
The MATLAB software plots an image showing the median pixel values for the foreground of the red (Cy5) channel.



- 2 Plot the median values for the green channel. For example, to plot data from the field F532 Median, type

```
figure  
mimage(pd, 'F532 Median')
```

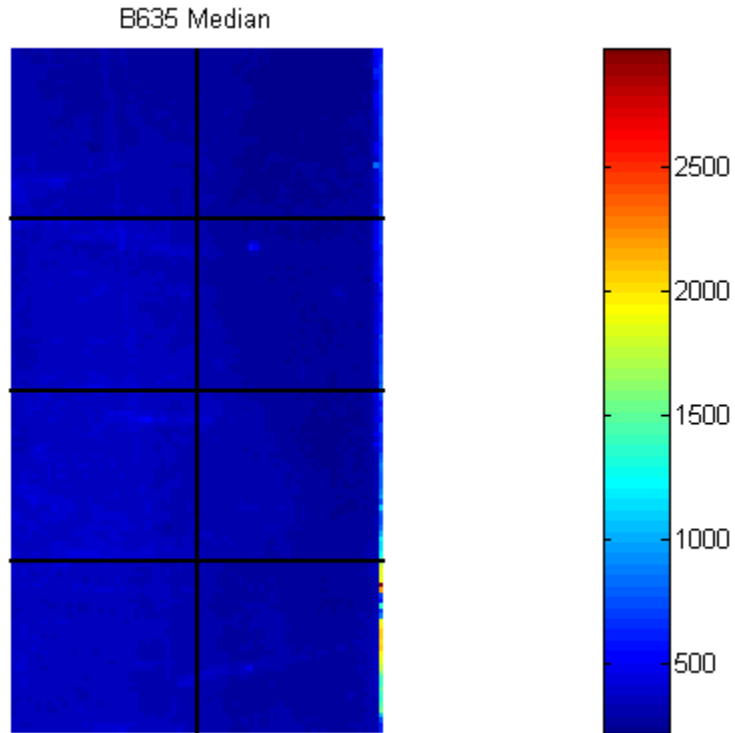
The MATLAB software plots an image showing the median pixel values of the foreground of the green (Cy3) channel.



- 3** Plot the median values for the red background. The field B635 Median shows the median values for the background of the red channel.

```
figure  
mimage(pd, 'B635 Median')
```

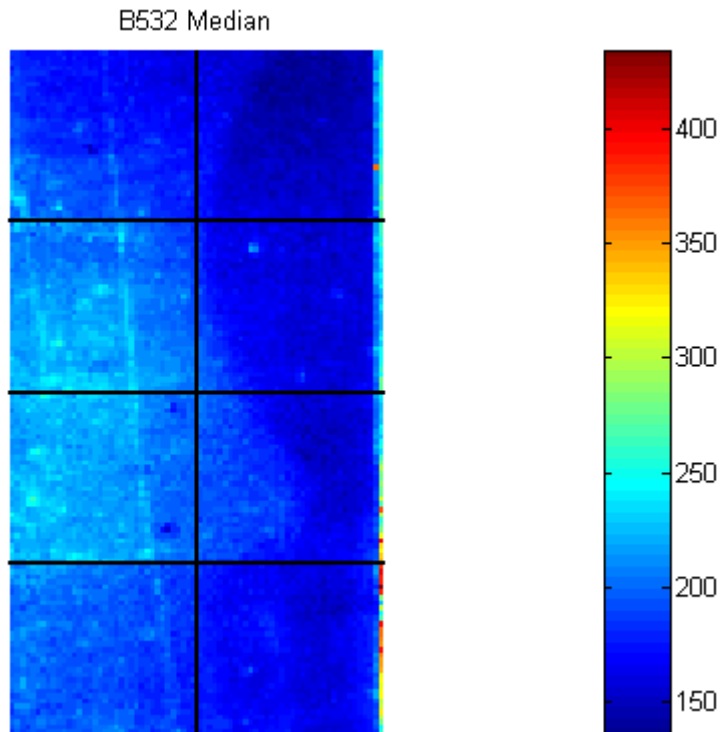
The MATLAB software plots an image for the background of the red channel. Notice the very high background levels down the right side of the array.



- 4** Plot the medial values for the green background. The field B532 Median shows the median values for the background of the green channel.

```
figure  
mimage(pd, 'B532 Median')
```

The MATLAB software plots an image for the background of the green channel.



- 5** The first array was for the Parkinson's disease model mouse. Now read in the data for the same brain voxel but for the untreated control mouse. In this case, the voxel sample was labeled with Cy3 and the control, total brain (not voxelated), was labeled with Cy5.

```
wt = gprread('mouse_a1wt.gpr')
```

The MATLAB software creates a structure and displays information about the structure.

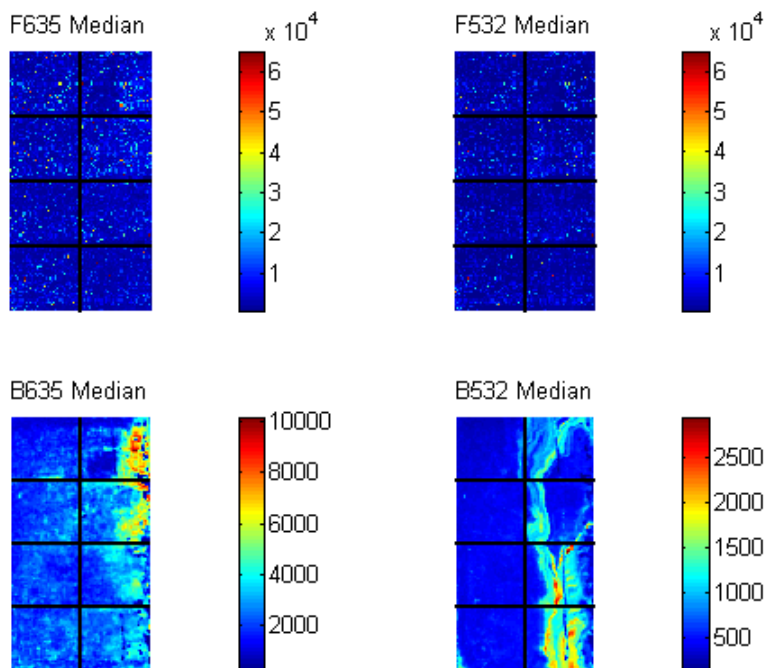


```
wt =  
    Header: [1x1 struct]  
    Data: [9504x38 double]  
    Blocks: [9504x1 double]  
    Columns: [9504x1 double]  
    Rows: [9504x1 double]  
    Names: {9504x1 cell}  
    IDs: {9504x1 cell}  
    ColumnNames: {38x1 cell}  
    Indices: [132x72 double]  
    Shape: [1x1 struct]
```

- 6 Use the function `mimage` to show pseudocolor images of the foreground and background. You can use the function `subplot` to put all the plots onto one figure.

```
figure  
subplot(2,2,1);  
mimage(wt, 'F635 Median')  
subplot(2,2,2);  
mimage(wt, 'F532 Median')  
subplot(2,2,3);  
mimage(wt, 'B635 Median')  
subplot(2,2,4);  
mimage(wt, 'B532 Median')
```

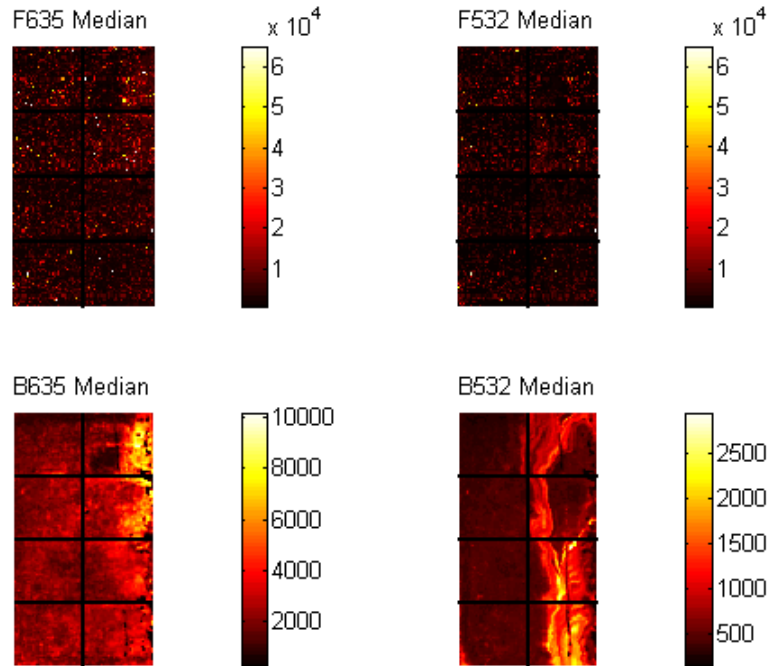
The MATLAB software plots the images.



- 7** If you look at the scale for the background images, you will notice that the background levels are much higher than those for the PD mouse and there appears to be something nonrandom affecting the background of the Cy3 channel of this slide. Changing the colormap can sometimes provide more insight into what is going on in pseudocolor plots. For more control over the color, try the `colormapeditor` function.

```
colormap hot
```

The MATLAB software plots the images.



- 8** The function `mimage` is a simple way to quickly create pseudocolor images of microarray data. However if you want more control over plotting, it is easy to create your own plots using the function `imagesc`.

First find the column number for the field of interest.

```
b532MedCol = find(strcmp(wt.ColumnNames, 'B532 Median'))
```

The MATLAB software displays:

```
b532MedCol =
    16
```

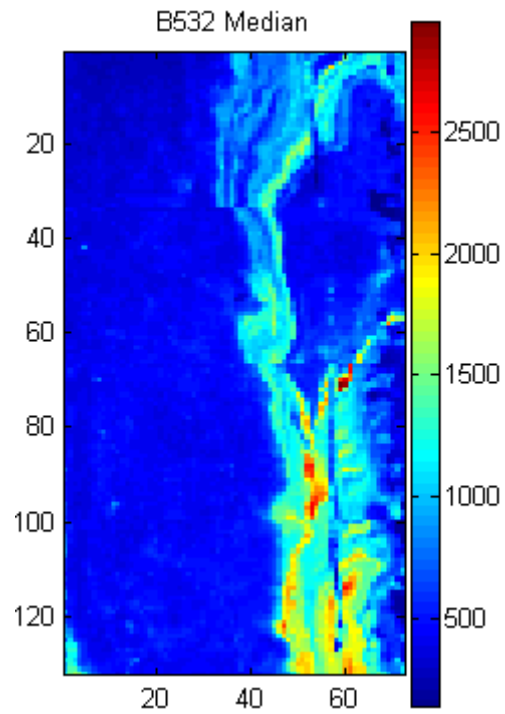
- 9** Extract that column from the field `Data`.

```
b532Data = wt.Data(:,b532MedCol);
```

**10** Use the field `Indices` to index into the `Data`.

```
figure
subplot(1,2,1);
imagesc(b532Data(wt.Indices))
axis image
colorbar
title('B532 Median')
```

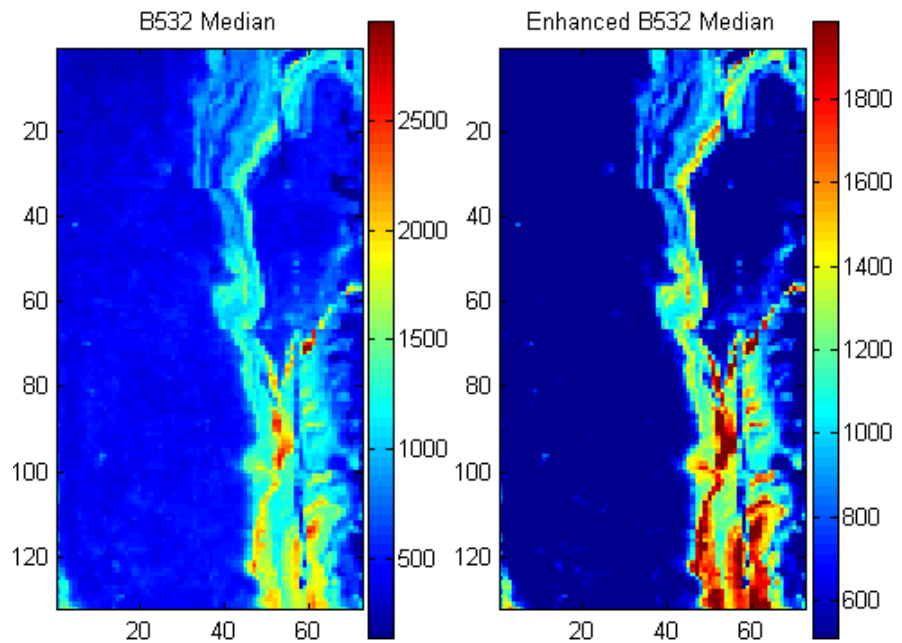
The MATLAB software plots the image.



- 11 Bound the intensities of the background plot to give more contrast in the image.

```
maskedData = b532Data;  
maskedData(b532Data<500) = 500;  
maskedData(b532Data>2000) = 2000;  
  
subplot(1,2,2);  
imagesc(maskedData(wt.Indices))  
axis image  
colorbar  
title('Enhanced B532 Median')
```

The MATLAB software plots the images.



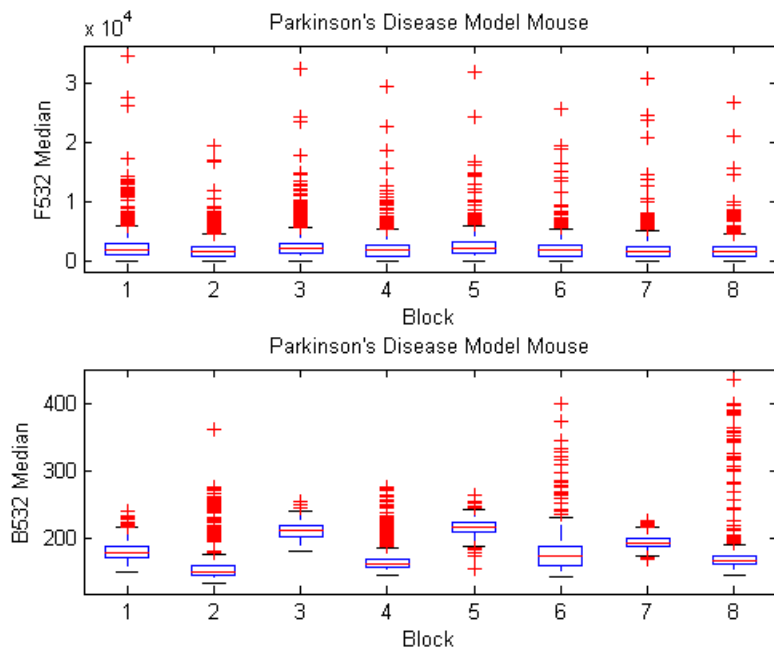
## Statistics of the Microarrays

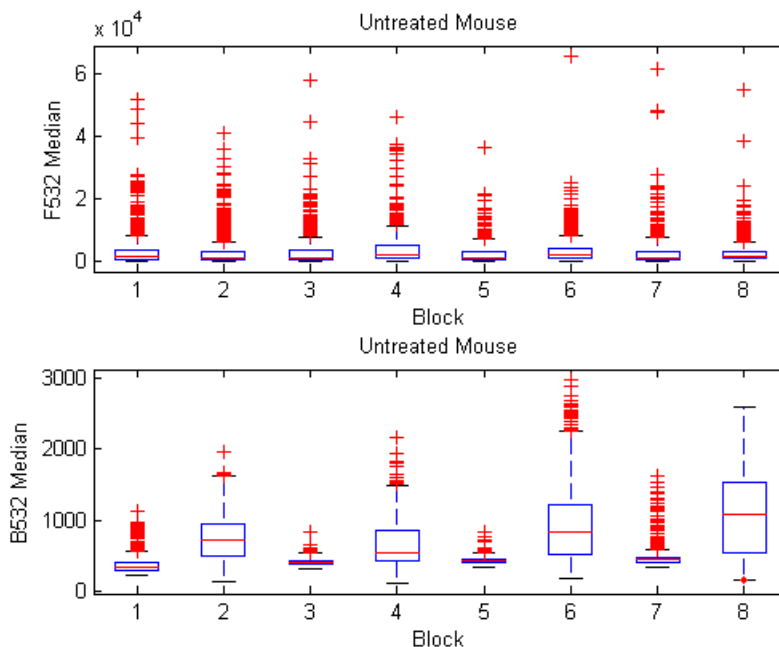
This procedure illustrates how to visualize distributions in microarray data. You can use the function `maboxplot` to look at the distribution of data in each of the blocks.

**1** In the MATLAB Command Window, type

```
figure
subplot(2,1,1)
maboxplot(pd, 'F532 Median', 'title', 'Parkinson' 's Disease Model Mouse')
subplot(2,1,2)
maboxplot(pd, 'B532 Median', 'title', 'Parkinson' 's Disease Model Mouse')
figure
subplot(2,1,1)
maboxplot(wt, 'F532 Median', 'title', 'Untreated Mouse')
subplot(2,1,2)
maboxplot(wt, 'B532 Median', 'title', 'Untreated Mouse')
```

The MATLAB software plots the images.





## 2 Compare the plots.

From the box plots you can clearly see the spatial effects in the background intensities. Blocks numbers 1, 3, 5, and 7 are on the left side of the arrays, and numbers 2, 4, 6, and 8 are on the right side. The data must be normalized to remove this spatial bias.

## Scatter Plots of Microarray Data

This procedure illustrates how to visualize expression levels in microarray data. There are two columns in the microarray data structure labeled 'F635 Median - B635' and 'F532 Median - B532'. These columns are the differences between the median foreground and the median background for the 635 nm channel and 532 nm channel respectively. These give a measure of the actual expression levels, although since the data must first be normalized to remove spatial bias in the background, you should be careful about using these values without further normalization. However, in this example no normalization is performed.



- 1 Rather than working with data in a larger structure, it is often easier to extract the column numbers and data into separate variables.

```
cy5DataCol = find(strcmp(wt.ColumnNames,'F635 Median - B635'))
cy3DataCol = find(strcmp(wt.ColumnNames,'F532 Median - B532'))
cy5Data = pd.Data(:,cy5DataCol);
cy3Data = pd.Data(:,cy3DataCol);
```

The MATLAB software displays:

```
cy5DataCol =
    34

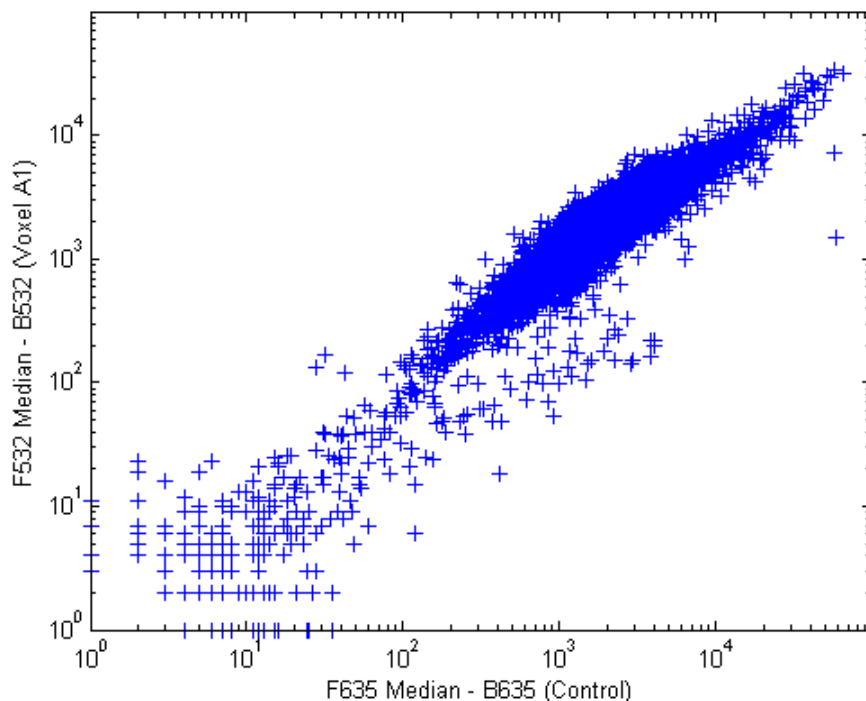
cy3DataCol =
    35
```

- 2 A simple way to compare the two channels is with a loglog plot. The function `maloglog` is used to do this. Points that are above the diagonal in this plot correspond to genes that have higher expression levels in the A1 voxel than in the brain as a whole.

```
figure
maloglog(cy5Data,cy3Data)
xlabel('F635 Median - B635 (Control)');
ylabel('F532 Median - B532 (Voxel A1)');
```

The MATLAB software displays the following messages and plots the images.

```
Warning: Zero values are ignored
(Type "warning off Bioinfo:MaloglogZeroValues" to suppress
this warning.)
Warning: Negative values are ignored.
(Type "warning off Bioinfo:MaloglogNegativeValues" to suppress
this warning.)
```



Notice that this function gives some warnings about negative and zero elements. This is because some of the values in the 'F635 Median - B635' and 'F532 Median - B532' columns are zero or even less than zero. Spots where this happened might be bad spots or spots that failed to hybridize. Points with positive, but very small, differences between foreground and background should also be considered to be bad spots.

- 3** Disable the display of warnings by using the `warning` command. Although warnings can be distracting, it is good practice to investigate why the warnings occurred rather than simply to ignore them. There might be some systematic reason why they are bad.

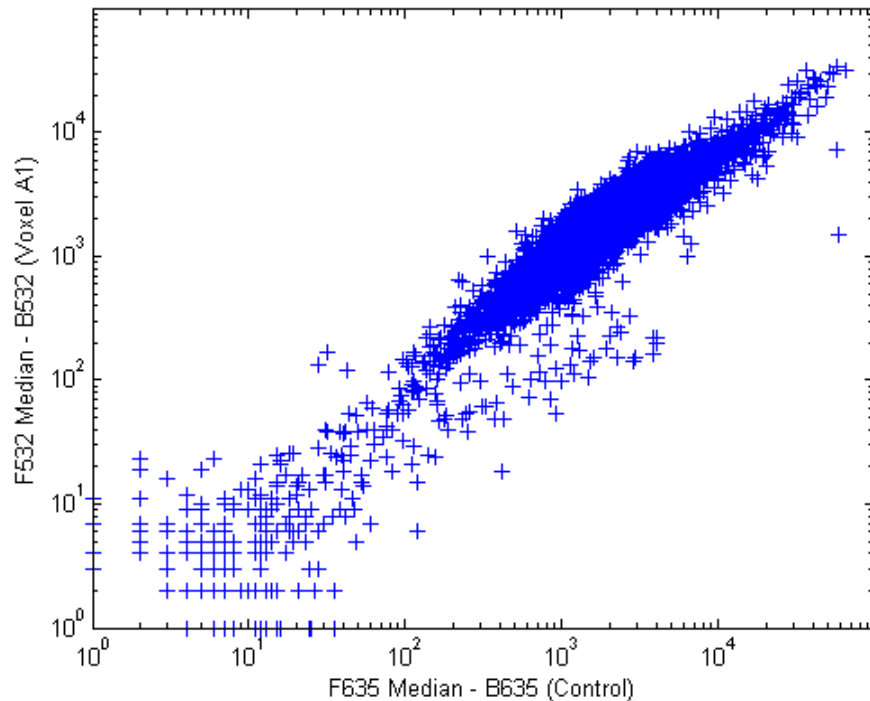
```
warnState = warning;           % First save the current warning
                               % state.
                               % Now turn off the two warnings.
warning('off','Bioinfo:MaloglogZeroValues');
warning('off','Bioinfo:MaloglogNegativeValues');
```

```

figure
maloglog(cy5Data,cy3Data)      % Create the loglog plot
warning(warnState);           % Reset the warning state.
xlabel('F635 Median - B635 (Control)');
ylabel('F532 Median - B532 (Voxel A1)');

```

The MATLAB software plots the image.



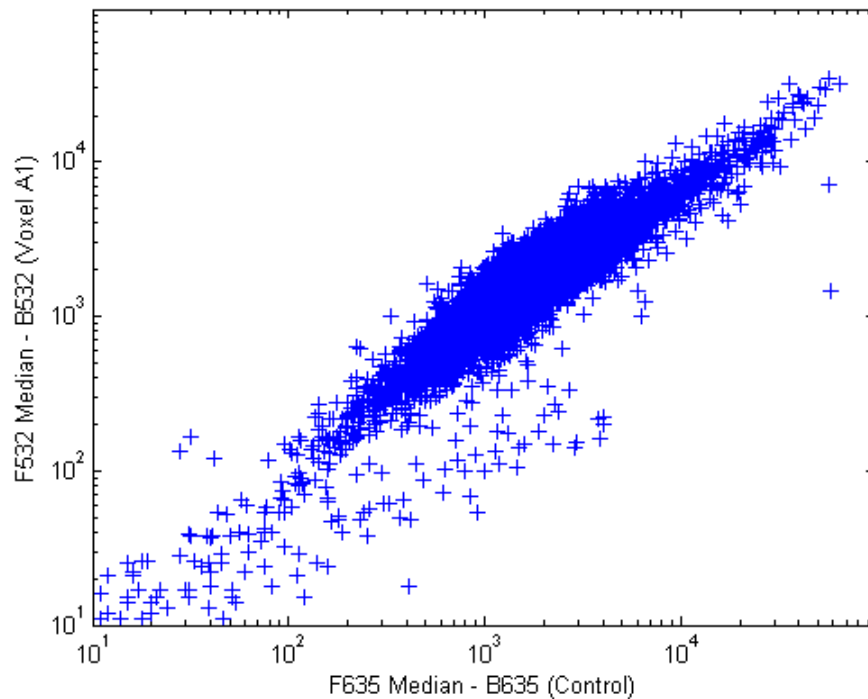
- 4** An alternative to simply ignoring or disabling the warnings is to remove the bad spots from the data set. You can do this by finding points where either the red or green channel has values less than or equal to a threshold value. For example, use a threshold value of 10.

```

threshold = 10;
badPoints = (cy5Data <= threshold) | (cy3Data <= threshold);

```

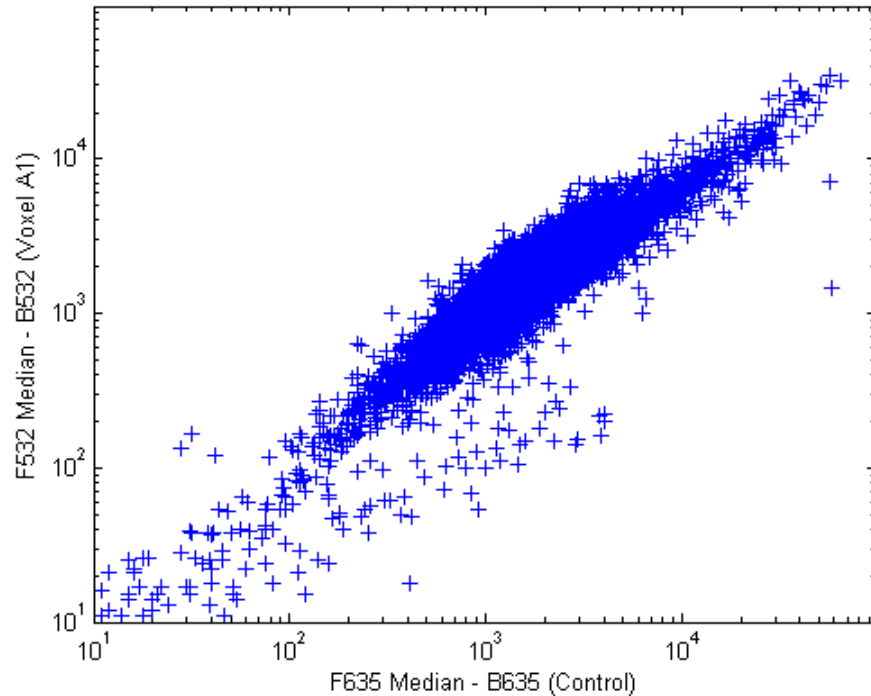
The MATLAB software plots the image.



5 You can then remove these points and redraw the loglog plot.

```
cy5Data(badPoints) = []; cy3Data(badPoints) = [];  
figure  
maloglog(cy5Data,cy3Data)  
xlabel('F635 Median - B635 (Control)');  
ylabel('F532 Median - B532 (Voxel A1)');
```

The MATLAB software plots the image.

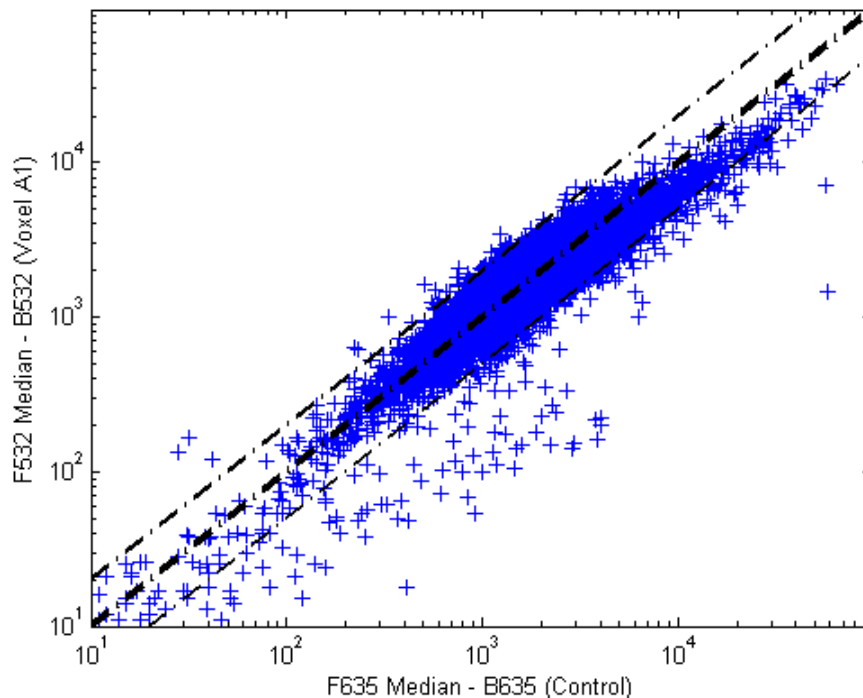


This plot shows the distribution of points but does not give any indication about which genes correspond to which points.

- 6 Add gene labels to the plot. Because some of the data points have been removed, the corresponding gene IDs must also be removed from the data set before you can use them. The simplest way to do that is `wt.IDs(~badPoints)`.

```
maloglog(cy5Data,cy3Data,'labels',wt.IDs(~badPoints),...
         'factorlines',2)
xlabel('F635 Median - B635 (Control)');
ylabel('F532 Median - B532 (Voxel A1)');
```

The MATLAB software plots the image.



**7** Try using the mouse to click some of the outlier points.

You will see the gene ID associated with the point. Most of the outliers are below the  $y = x$  line. In fact, most of the points are below this line. Ideally the points should be evenly distributed on either side of this line.

**8** Normalize the points to evenly distribute them on either side of the line. Use the function `mameannorm` to perform global mean normalization.

```
normcy5 = mameannorm(cy5Data);
normcy3 = mameannorm(cy3Data);
```

If you plot the normalized data you will see that the points are more evenly distributed about the  $y = x$  line.

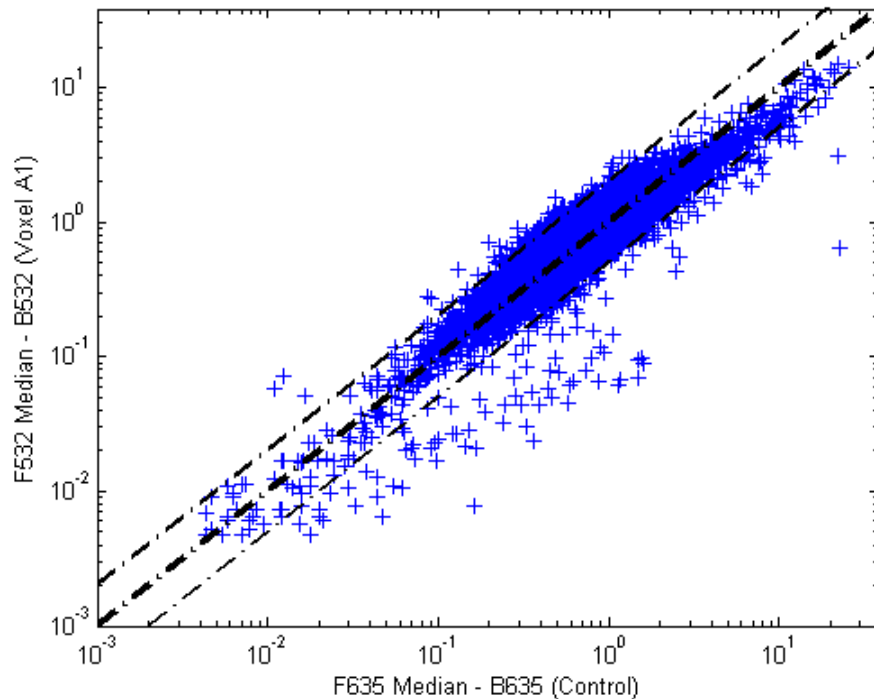
```
figure
```

```

maloglog(normcy5,normcy3,'labels',wt.IDs(~badPoints),...
         'factorlines',2)
xlabel('F635 Median - B635 (Control)');
ylabel('F532 Median - B532 (Voxel A1)');

```

The MATLAB software plots the image.



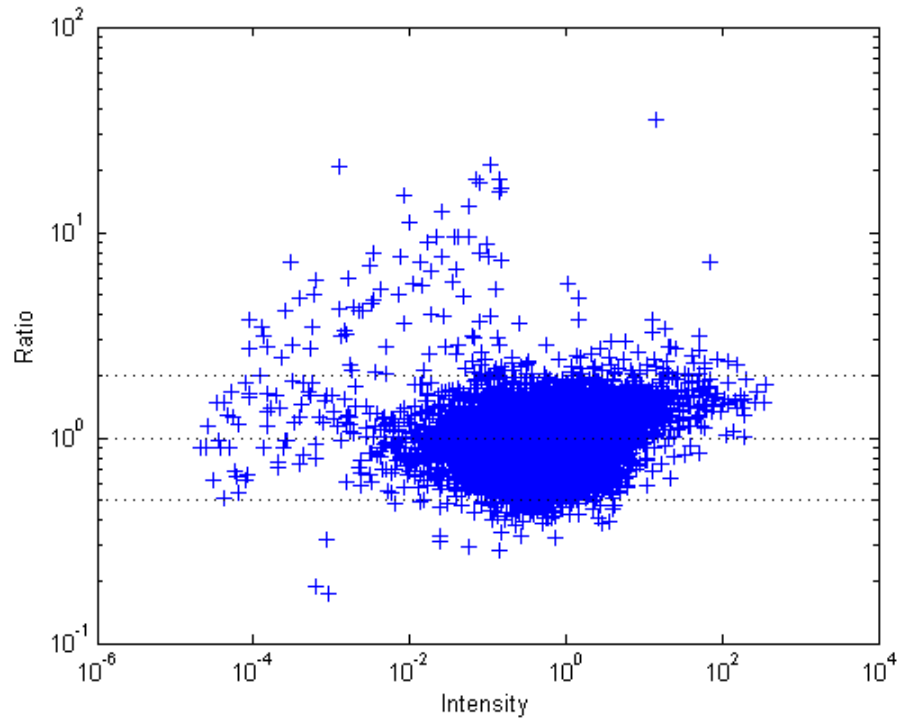
- 9 The function `mairplot` is used to create an Intensity vs. Ratio plot for the normalized data. This function works in the same way as the function `maloglog`.

```

figure
mairplot(normcy5,normcy3,'labels',wt.IDs(~badPoints),...
         'factorlines',2)

```

The MATLAB software plots the image.



- 10** You can click the points in this plot to see the name of the gene associated with the plot.



## Example: Analyzing Gene Expression Profiles

### In this section...

“Overview of the Yeast Example” on page 3-57

“Exploring the Data Set” on page 3-57

“Filtering Genes” on page 3-61

“Clustering Genes” on page 3-64

“Principal Component Analysis” on page 3-68

### Overview of the Yeast Example

This example demonstrates a number of ways to look for patterns in gene expression profiles, using gene expression data from yeast shifting from fermentation to respiration.

The microarray data for this example is from DeRisi, J.L., Iyer, V.R., and Brown, P.O. (Oct 24, 1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science*, 278 (5338), 680–686. PMID: 9381177.

The authors used DNA microarrays to study temporal gene expression of almost all genes in *Saccharomyces cerevisiae* during the metabolic shift from fermentation to respiration. Expression levels were measured at seven time points during the diauxic shift. The full data set can be downloaded from the Gene Expression Omnibus Web site at:

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE28>

### Exploring the Data Set

This procedure illustrates how to import data from the Web into the MATLAB environment. The data for this procedure is available in the MAT-file `yeastdata.mat`. This file contains the VALUE data or LOG\_RAT2N\_MEAN, or log2 of ratio of CH2DN\_MEAN and CH1DN\_MEAN from the seven time steps in the experiment, the names of the genes, and an array of the times at which the expression levels were measured.

1 Load data into the MATLAB environment.

```
load yeastdata.mat
```

- 2** Get the size of the data by typing

```
numel(genes)
```

The number of genes in the data set displays in the MATLAB Command Window. The MATLAB variable `genes` is a cell array of the gene names.

```
ans =  
    6400
```

- 3** Access the entries using cell array indexing.

```
genes{15}
```

This displays the 15th row of the variable `yeastvalues`, which contains expression levels for the open reading frame (ORF) YAL054C.

```
ans =  
    YAL054C
```

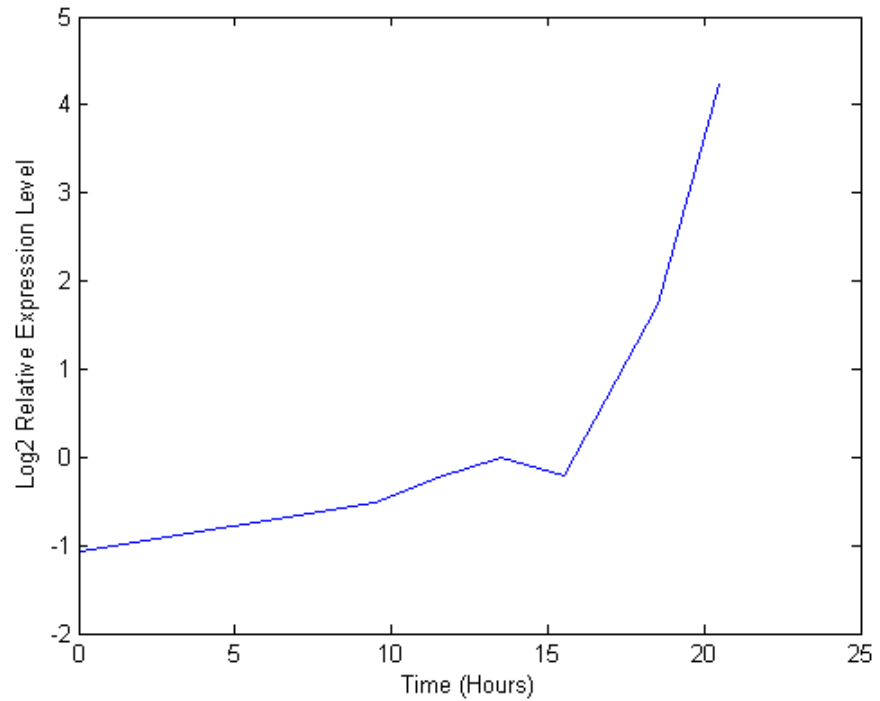
- 4** Use the function `web` to access information about this ORF in the Saccharomyces Genome Database (SGD).

```
url = sprintf(...  
    'http://genome-www4.stanford.edu/cgi-bin/SGD/...  
    locus.pl?locus=%s',...  
    genes{15});  
web(url);
```

- 5** A simple plot can be used to show the expression profile for this ORF.

```
plot(times, yeastvalues(15,:))  
xlabel('Time (Hours)');  
ylabel('Log2 Relative Expression Level');
```

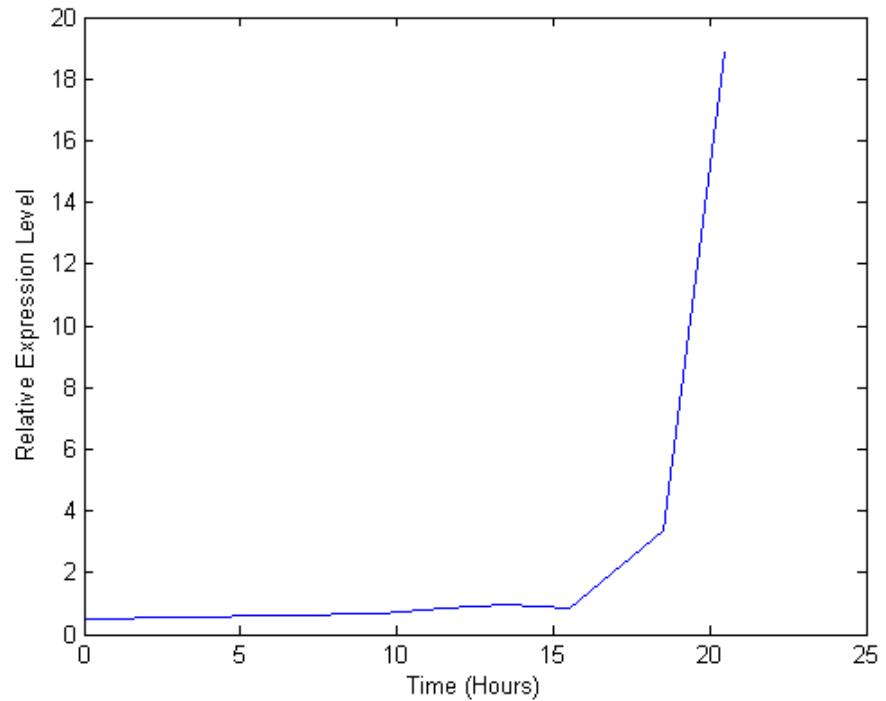
The MATLAB software plots the figure. The values are  $\log_2$  ratios.



**6** Plot the actual values.

```
plot(times, 2.^yeastvalues(15,:))  
xlabel('Time (Hours)');  
ylabel('Relative Expression Level');
```

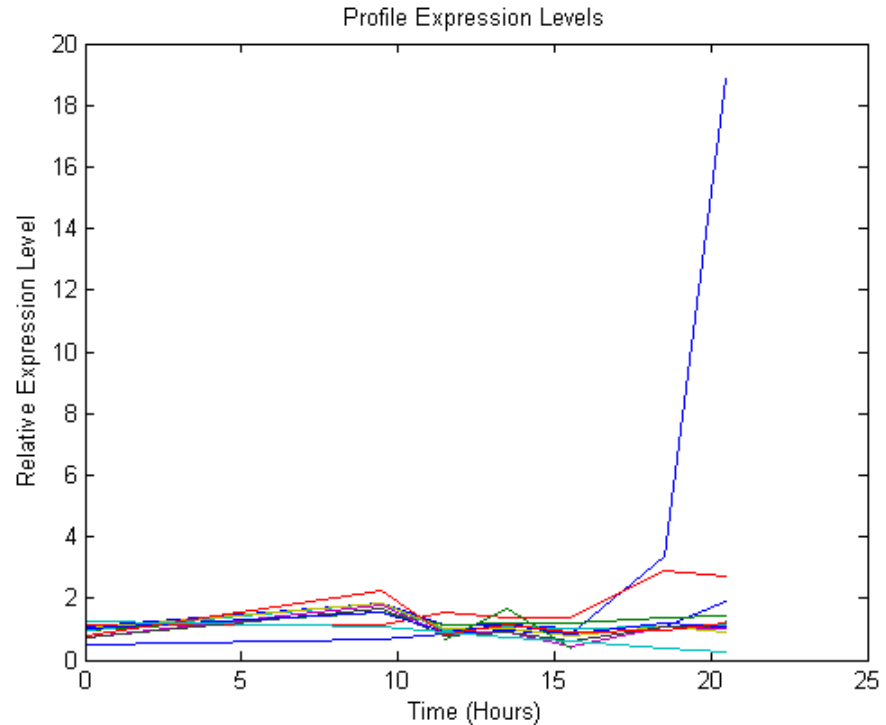
The MATLAB software plots the figure. The gene associated with this ORF, ACS1, appears to be strongly up-regulated during the diauxic shift.



**7** Compare other genes by plotting multiple lines on the same figure.

```
hold on
plot(times, 2.^yeastvalues(16:26,:))
xlabel('Time (Hours)');
ylabel('Relative Expression Level');
title('Profile Expression Levels');
```

The MATLAB software plots the image.



## Filtering Genes

This procedure illustrates how to filter the data by removing genes that are not expressed or do not change. The data set is quite large and a lot of the information corresponds to genes that do not show any interesting changes during the experiment. To make it easier to find the interesting genes, reduce the size of the data set by removing genes with expression profiles that do not show anything of interest. There are 6400 expression profiles. You can use a number of techniques to reduce the number of expression profiles to some subset that contains the most significant genes.

- 1 If you look through the gene list you will see several spots marked as 'EMPTY'. These are empty spots on the array, and while they might have data associated with them, for the purposes of this example, you can

consider these points to be noise. These points can be found using the `strcmp` function and removed from the data set with indexing commands.

```
emptySpots = strcmp('EMPTY',genes);
yeastvalues(emptySpots,:) = [];
genes(emptySpots) = [];
numel(genes)
```

The MATLAB software displays:

```
ans =
    6314
```

In the `yeastvalues` data you will also see several places where the expression level is marked as `NaN`. This indicates that no data was collected for this spot at the particular time step. One approach to dealing with these missing values would be to impute them using the mean or median of data for the particular gene over time. This example uses a less rigorous approach of simply throwing away the data for any genes where one or more expression levels were not measured.

- 2 Use the `isnan` function to identify the genes with missing data and then use indexing commands to remove the genes.

```
nanIndices = any(isnan(yeastvalues),2);
yeastvalues(nanIndices,:) = [];
genes(nanIndices) = [];
numel(genes)
```

The MATLAB software displays:

```
ans =
    6276
```

If you were to plot the expression profiles of all the remaining profiles, you would see that most profiles are flat and not significantly different from the others. This flat data is obviously of use as it indicates that the genes associated with these profiles are not significantly affected by the diauxic shift. However, in this example, you are interested in the genes with large changes in expression accompanying the diauxic shift. You can use filtering functions in the toolbox to remove genes with various types

of profiles that do not provide useful information about genes affected by the metabolic change.

- 3** Use the function `genevarfilter` to filter out genes with small variance over time. The function returns a logical array of the same size as the variable `genes` with ones corresponding to rows of `yeastvalues` with variance greater than the 10th percentile and zeros corresponding to those below the threshold.

```
mask = genevarfilter(yeastvalues);
% Use the mask as an index into the values to remove the
% filtered genes.
yeastvalues = yeastvalues(mask,:);
genes = genes(mask);
numel(genes)
```

The MATLAB software displays:

```
ans =
    5648
```

- 4** The function `genelowvalfilter` removes genes that have very low absolute expression values. Note that the gene filter functions can also automatically calculate the filtered data and names.

```
[mask, yeastvalues, genes] = genelowvalfilter(yeastvalues,genes,...
                                              'absval',log2(4));
numel(genes)
```

The MATLAB software displays:

```
ans =
    423
```

- 5** Use the function `geneentropyfilter` to remove genes whose profiles have low entropy:

```
[mask, yeastvalues, genes] = geneentropyfilter(yeastvalues,genes,...
                                              'prctile',15);
numel(genes)
```

The MATLAB software displays:

```
ans = 310
```

## Clustering Genes

Now that you have a manageable list of genes, you can look for relationships between the profiles using some different clustering techniques from the Statistics Toolbox software.

- 1** For hierarchical clustering, the function `pdist` calculates the pairwise distances between profiles, and the function `linkage` creates the hierarchical cluster tree.

```
corrDist = pdist(yeastvalues, 'corr');  
clusterTree = linkage(corrDist, 'average');
```

- 2** The function `cluster` calculates the clusters based on either a cutoff distance or a maximum number of clusters. In this case, the `'maxclust'` option is used to identify 16 distinct clusters.

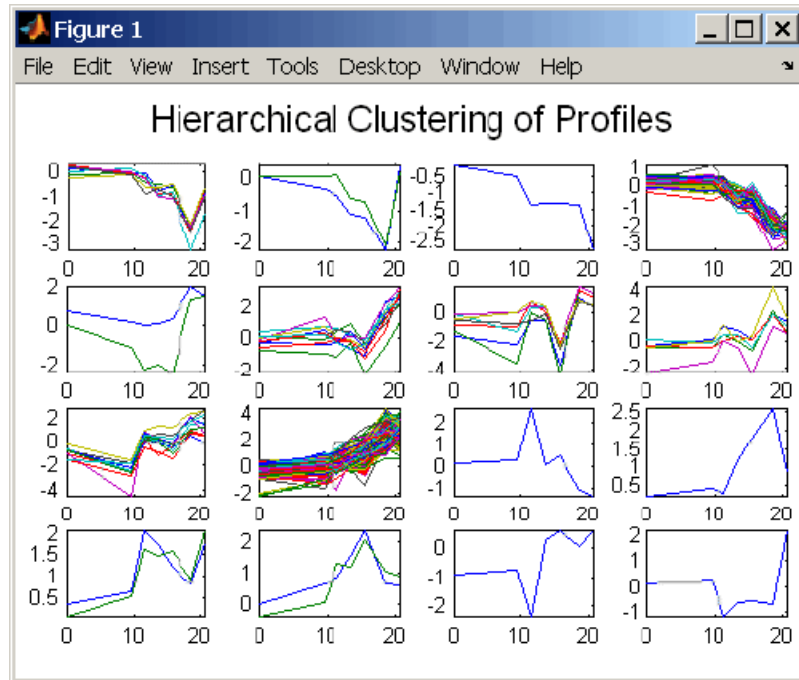
```
clusters = cluster(clusterTree, 'maxclust', 16);
```

- 3** The profiles of the genes in these clusters can be plotted together using a simple loop and the function `subplot`.

```
figure  
for c = 1:16  
    subplot(4,4,c);  
    plot(times,yeastvalues((clusters == c),:));  
    axis tight  
end  
suptitle('Hierarchical Clustering of Profiles');
```

The MATLAB software plots the images.





- 4 The Statistics Toolbox software also has a K-means clustering function. Again, 16 clusters are found, but because the algorithm is different these are not necessarily the same clusters as those found by hierarchical clustering.

```
[cidx, ctrs] = kmeans(yeastvalues, 16,...
                    'dist','corr',...
                    'rep',5,...
                    'disp','final');

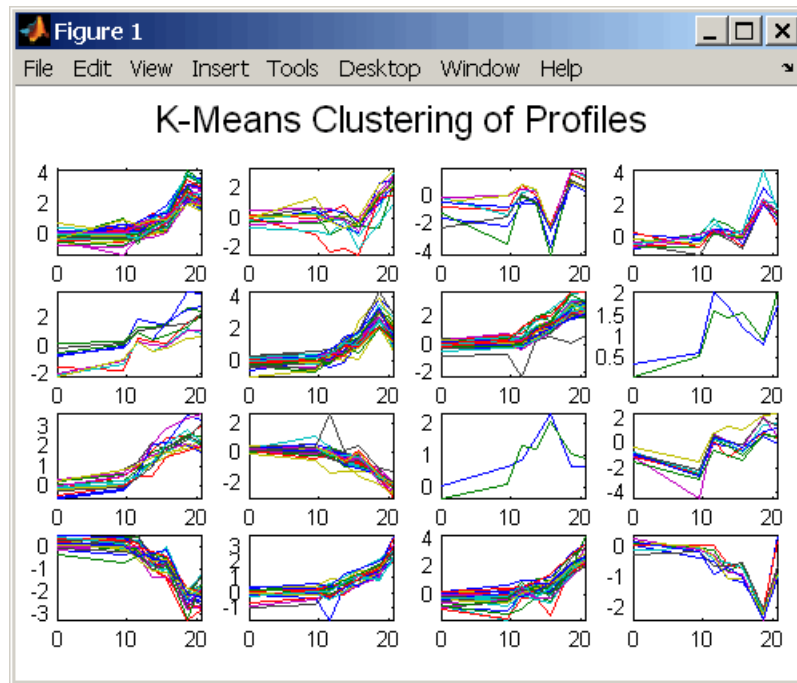
figure
for c = 1:16
    subplot(4,4,c);
    plot(times,yeastvalues((cidx == c),:));
    axis tight
end
suptitle('K-Means Clustering of Profiles');
```

The MATLAB software displays:

```

13 iterations, total sum of distances = 11.4042
14 iterations, total sum of distances = 8.62674
26 iterations, total sum of distances = 8.86066
22 iterations, total sum of distances = 9.77676
26 iterations, total sum of distances = 9.01035

```



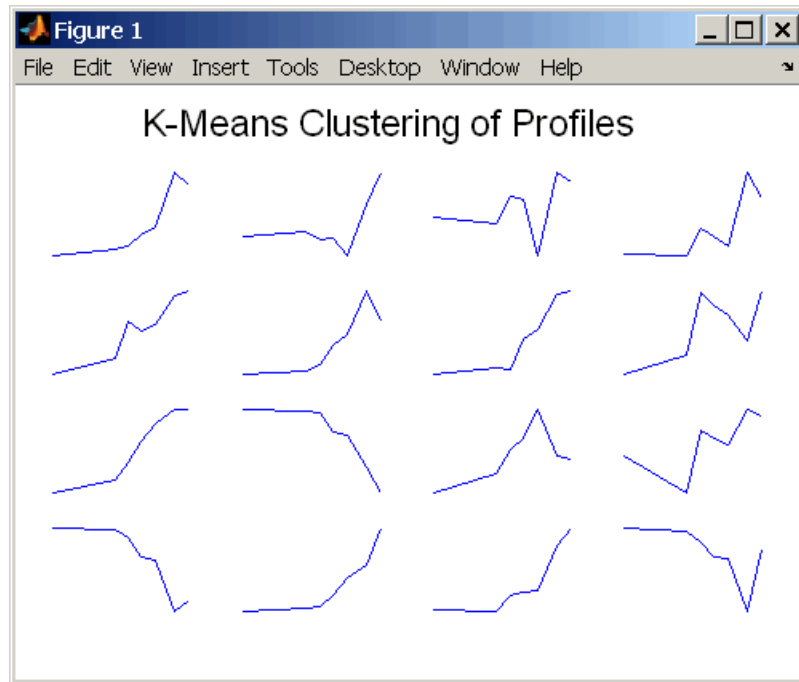
5 Instead of plotting all of the profiles, you can plot just the centroids.

```

figure
for c = 1:16
    subplot(4,4,c);
    plot(times, ctrs(c,:));
    axis tight
    axis off    % turn off the axis
end
suptitle('K-Means Clustering of Profiles');

```

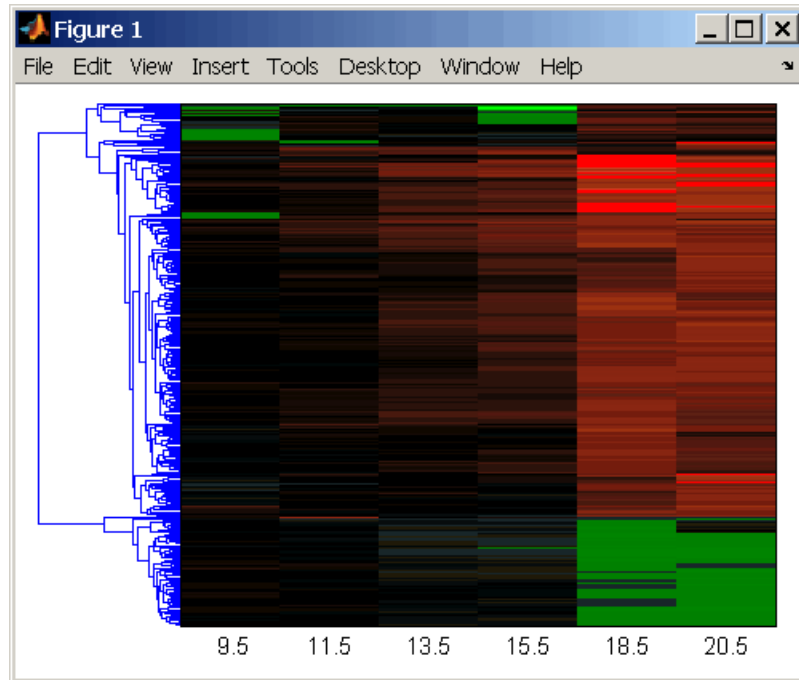
The MATLAB software plots the figure:



- 6 You can use the function `clustergram` to create a heat map and dendrogram from the output of the hierarchical clustering.

```
figure
clustergram(yeastvalues(:,2:end), 'RowLabels', genes, ...
            'ColumnLabels', times(2:end))
```

The MATLAB software plots the figure:



## Principal Component Analysis

Principal-component analysis (PCA) is a useful technique you can use to reduce the dimensionality of large data sets, such as those from microarray analysis. You can also use PCA to find signals in noisy data.

- 1 Use the `princomp` function in the Statistics Toolbox software to calculate the principal components of a data set.

```
[pc, zscores, pcvars] = princomp(yeastvalues)
```

The MATLAB software displays:

```
pc =
```

```
Columns 1 through 4
```

```

-0.0245  -0.3033  -0.1710  -0.2831
 0.0186  -0.5309  -0.3843  -0.5419
 0.0713  -0.1970   0.2493   0.4042
 0.2254  -0.2941   0.1667   0.1705
 0.2950  -0.6422   0.1415   0.3358
 0.6596   0.1788   0.5155  -0.5032
 0.6490   0.2377  -0.6689   0.2601

```

Columns 5 through 7

```

-0.1155   0.4034   0.7887
-0.2384  -0.2903  -0.3679
-0.7452  -0.3657   0.2035
-0.2385   0.7520  -0.4283
 0.5592  -0.2110   0.1032
-0.0194  -0.0961   0.0667
-0.0673  -0.0039   0.0521

```

- 2** You can use the function `cumsum` to see the cumulative sum of the variances.

```
cumsum(pcvvars ./ sum(pcvvars) * 100)
```

The MATLAB software displays:

```

ans =
 78.3719
 89.2140
 93.4357
 96.0831
 98.3283
 99.3203
100.0000

```

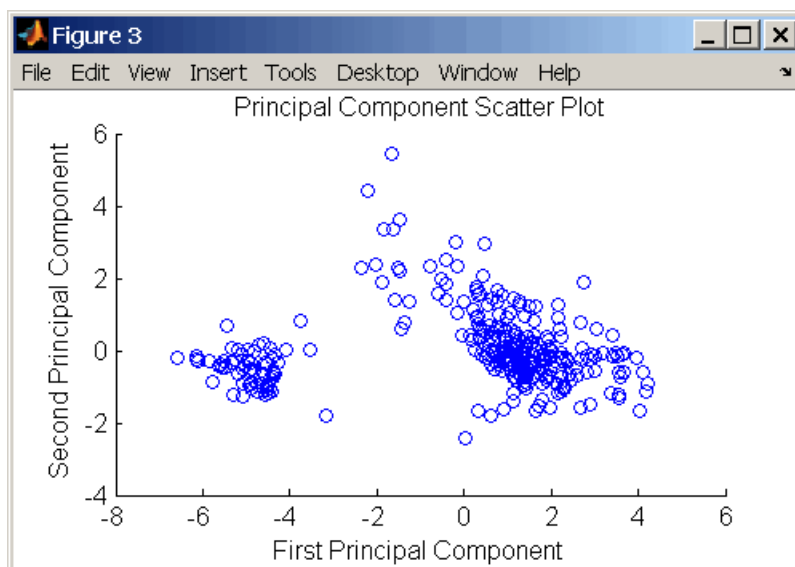
This shows that almost 90% of the variance is accounted for by the first two principal components.

- 3** A scatter plot of the scores of the first two principal components shows that there are two distinct regions. This is not unexpected, because the filtering

process removed many of the genes with low variance or low information. These genes would have appeared in the middle of the scatter plot.

```
figure
scatter(zscores(:,1),zscores(:,2));
xlabel('First Principal Component');
ylabel('Second Principal Component');
title('Principal Component Scatter Plot');
```

The MATLAB software plots the figure:



- 4 The `gname` function from the Statistics Toolbox software can be used to identify genes on a scatter plot. You can select as many points as you like on the scatter plot.

```
gname(genes);
```

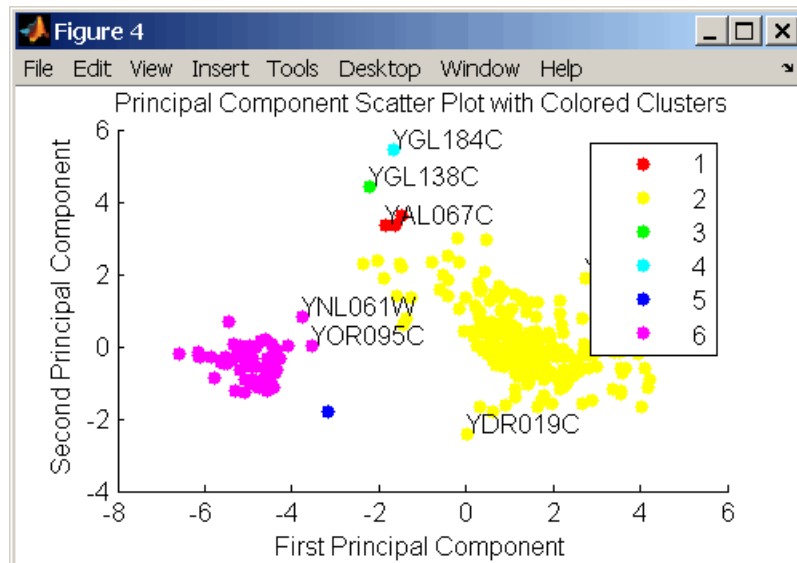
When you have finished selecting points, press **Enter**.

- 5 An alternative way to create a scatter plot is with the `gscatter` function from the Statistics Toolbox software. `gscatter` creates a grouped scatter

plot where points from each group have a different color or marker. You can use `clusterdata`, or any other clustering function, to group the points.

```
figure
pcclusters = clusterdata(zscores(:,1:2),6);
gscatter(zscores(:,1),zscores(:,2),pcclusters)
xlabel('First Principal Component');
ylabel('Second Principal Component');
title('Principal Component Scatter Plot with Colored Clusters');
gname(genes) % Press enter when you finish selecting genes.
```

The MATLAB software plots the figure:







# Phylogenetic Analysis

---

- “Overview of Phylogenetic Analysis” on page 4-2
- “Example: Building a Phylogenetic Tree” on page 4-3
- “Phylogenetic Tree Tool Reference” on page 4-16

# Overview of Phylogenetic Analysis

Phylogenetic analysis is the process you use to determine the evolutionary relationships between organisms. The results of an analysis can be drawn in a hierarchical diagram called a cladogram or phylogram (phylogenetic tree). The branches in a tree are based on the hypothesized evolutionary relationships (phylogeny) between organisms. Each member in a branch, also known as a monophyletic group, is assumed to be descended from a common ancestor. Originally, phylogenetic trees were created using morphology, but now, determining evolutionary relationships includes matching patterns in nucleic acid and protein sequences.

## Example: Building a Phylogenetic Tree

In this section...
“Overview of the Primate Example” on page 4-3
“Searching NCBI for Phylogenetic Data” on page 4-5
“Creating a Phylogenetic Tree for Five Species” on page 4-6
“Creating a Phylogenetic Tree for Twelve Species” on page 4-9
“Exploring the Phylogenetic Tree” on page 4-11

---

**Note** For information on creating a phylogenetic tree with multiply aligned sequences, see the `phytree` function.

---

### Overview of the Primate Example

In this example, a phylogenetic tree is constructed from mitochondrial DNA (mtDNA) sequences for the family Hominidae. This family includes gorillas, chimpanzees, orangutans, and humans.

The following procedures demonstrate the phylogenetic analysis features in the Bioinformatics Toolbox software. They are not intended to teach the process of phylogenetic analysis, but to show you how to use MathWorks products to create a phylogenetic tree from a set of nonaligned nucleotide sequences.

The origin of modern humans is a heavily debated issue that scientists have recently tackled by using mitochondrial DNA (mtDNA) sequences. One hypothesis explains the limited genetic variation of human mtDNA in terms of a recent common genetic ancestry, implying that all modern population mtDNA originated from a single woman who lived in Africa less than 200,000 years ago.

### Why Use Mitochondrial DNA Sequences for Phylogenetic Study?

Mitochondrial DNA sequences, like the Y chromosome, do not recombine and are inherited from the maternal parent. This lack of recombination

allows sequences to be traced through one genetic line and all polymorphisms assumed to be caused by mutations.

Mitochondrial DNA in mammals has a faster mutation rate than nuclear DNA sequences. This faster rate of mutation produces more variance between sequences and is an advantage when studying closely related species. The mitochondrial control region (Displacement or D-loop) is one of the fastest mutating sequence regions in animal DNA.

### **Neanderthal DNA**

The ability to isolate mitochondrial DNA (mtDNA) from palaeontological samples has allowed genetic comparisons between extinct species and closely related nonextinct species. The reasons for isolating mtDNA instead of nuclear DNA in fossil samples have to do with the fact that:

- mtDNA, because it is circular, is more stable and degrades slower than nuclear DNA.
- Each cell can contain a thousand copies of mtDNA and only a single copy of nuclear DNA.

While there is still controversy as to whether Neanderthals are direct ancestors of humans or evolved independently, the use of ancient genetic sequences in phylogenetic analysis adds an interesting dimension to the question of human ancestry.

### **References**

Ovchinnikov I., et al. (2000). Molecular analysis of Neanderthal DNA from the northern Caucasus. *Nature* 404(6777), 490–493.

Sajantila A., et al. (1995). Genes and languages in Europe: an analysis of mitochondrial lineages. *Genome Research* 5 (1), 42–52.

Krings M., et al. (1997). Neanderthal DNA sequences and the origin of modern humans. *Cell* 90 (1), 19–30.

Jensen-Seaman, M., Kidd K. (2001). Mitochondrial DNA variation and biogeography of eastern gorillas. *Molecular Ecology* 10(9), 2241–2247.

## Searching NCBI for Phylogenetic Data

The NCBI taxonomy Web site includes phylogenetic and taxonomic information from many sources. These sources include the published literature, Web databases, and taxonomy experts. And while the NCBI taxonomy database is not a phylogenetic or taxonomic authority, it can be useful as a gateway to the NCBI biological sequence databases.

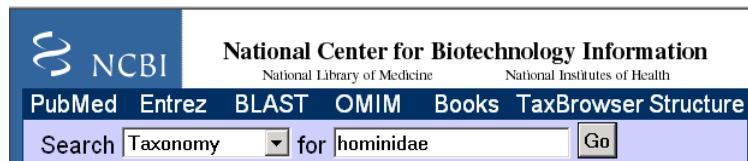
This procedure uses the family Hominidae (orangutans, chimpanzees, gorillas, and humans) as a taxonomy example for searching the NCBI Web site and locating mitochondrial D-loop sequences.

- 1 Use the MATLAB Help browser to search for data on the Web. In the MATLAB Command Window, type

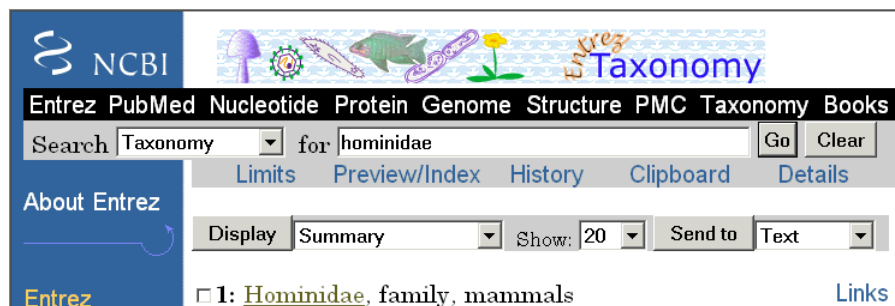
```
web('http://www.ncbi.nlm.nih.gov')
```

A separate browser window opens with the home page for the NCBI Web site.

- 2 Search the NCBI Web site for information. For example, to search for the human taxonomy, from the **Search** list, select **Taxonomy**, and in the **for** box, enter **hominidae**.



The NCBI Web search returns a list of links to relevant pages.



- 3 Select the taxonomy link for the family Hominidae. A page with the taxonomy for the family is shown.

NCBI Taxonomy Browser

Entrez PubMed Nucleotide Protein Genome Structure PMC Taxonomy Books

Search for \_\_\_\_\_ as   lock

Display  levels using filter:

Nucleotide  Protein  Structure  Genome  Popset  SNP

3D Domains  Domains  GEO  Expressions  UniGene  UniSTS

PubMed Central  Gene  MapView  LinkOut  BLAST  TRACE

[Lineage](#) (full): [root](#); [cellular organisms](#); [Eukaryota](#); [Fungi/Metazoa group](#); [Metazoa](#); [Eumetazoa](#); [Bilateria](#); [Coelomata](#); [Deuterostomia](#); [Chordata](#); [Craniata](#); [Vertebrata](#); [Gnathostomata](#); [Teleostomi](#); [Euteleostomi](#); [Sarcopterygii](#); [Tetrapoda](#); [Amniota](#); [Mammalia](#); [Theria](#); [Eutheria](#); [Primates](#); [Catarrhini](#)

- o [Hominidae](#) *Click on organism name to get more information.*
  - o [Homo/Pan/Gorilla group](#)
    - o [Gorilla](#)
      - [Gorilla gorilla](#) (gorilla)
    - o [Homo](#)
      - [Homo sapiens](#) (human)
    - o [Pan](#) (chimpanzees)
      - [Pan paniscus](#) (pygmy chimpanzee)
      - [Pan troglodytes](#) (chimpanzee)
    - o [Pongo](#)
      - o [Pongo pygmaeus](#) (orangutan)
        - [Pongo pygmaeus abelii](#) (Sumatran orangutan)
        - [Pongo pygmaeus pygmaeus](#) (Bornean orangutan)
      - [Pongo sp.](#)

## Creating a Phylogenetic Tree for Five Species

Drawing a phylogenetic tree using sequence data is helpful when you are trying to visualize the evolutionary relationships between species. The sequences can be multiply aligned or a set of nonaligned sequences, you can select a method for calculating pairwise distances between sequences, and

you can select a method for calculating the hierarchical clustering distances used to build a tree.

After locating the GenBank accession codes for the sequences you are interested in studying, you can create a phylogenetic tree with the data. For information on locating accession codes, see “Searching NCBI for Phylogenetic Data” on page 4-5.

In the following example, you will use the Jukes-Cantor method to calculate distances between sequences, and the Unweighted Pair Group Method Average (UPGMA) method for linking the tree nodes.

- 1** Create a MATLAB structure with information about the sequences. This step uses the accession codes for the mitochondrial D-loop sequences isolated from different hominid species.

```
data = {'German_Neanderthal'      'AF011222';
        'Russian_Neanderthal'    'AF254446';
        'European_Human'        'X90314';
        'Mountain_Gorilla_Rwanda' 'AF089820';
        'Chimp_Troglodytes'      'AF176766';
        };
```

- 2** Retrieve sequence data from the GenBank database and copy into the MATLAB environment.

```
for ind = 1:5
    seqs(ind).Header = data{ind,1};
    seqs(ind).Sequence = getgenbank(data{ind,2},...
                                    'sequenceonly', true);
end
```

- 3** Calculate pairwise distances and create a phytree object. For example, compute the pairwise distances using the Jukes-Cantor distance method and build a phylogenetic tree using the UPGMA linkage method. Since the sequences are not prealigned, `seqpdist` pairwise aligns them before computing the distances.

```
distances = seqpdist(seqs,'Method','Jukes-Cantor','Alphabet','DNA');
tree = seqlinkage(distances,'UPGMA',seqs)
```

The MATLAB software displays information about the `phytree` object. The function `seqpdist` calculates the pairwise distances between pairs of sequences while the function `seqlinkage` uses the distances to build a hierarchical cluster tree. First, the most similar sequences are grouped together, and then sequences are added to the tree in descending order of similarity.

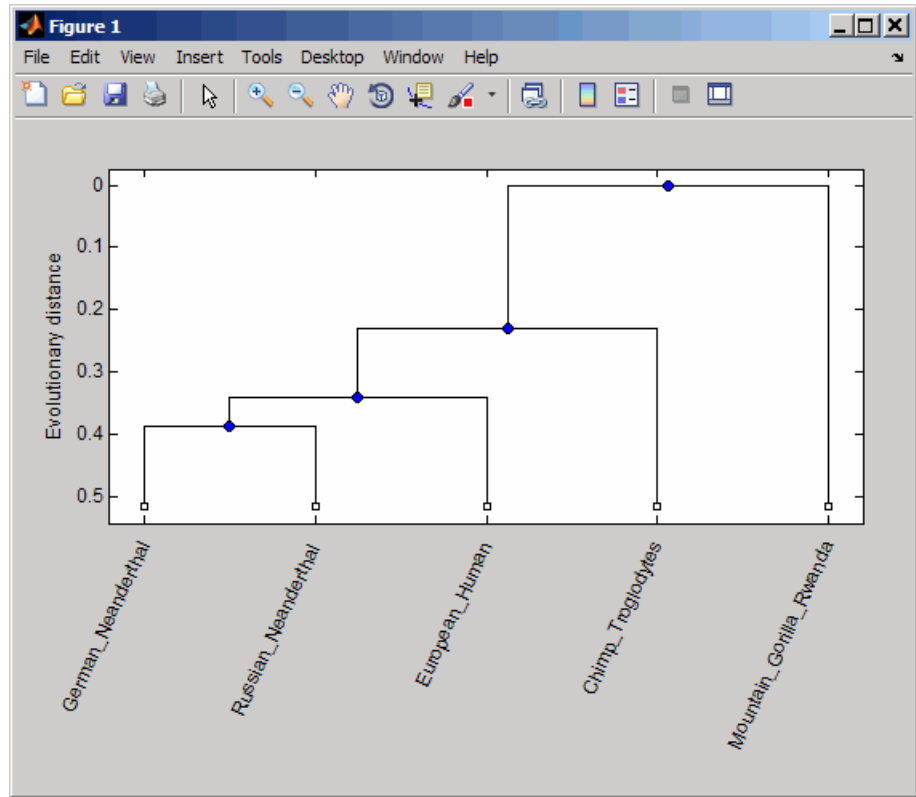
Phylogenetic tree object with 5 leaves (4 branches)

### 4 Draw a phylogenetic tree.

```
h = plot(tree,'orient','top');  
ylabel('Evolutionary distance')  
set(h.terminalNodeLabels,'Rotation',65)
```

The MATLAB software draws a phylogenetic tree in a Figure window. In the figure below, the hypothesized evolutionary relationships between the species is shown by the location of species on the branches. The horizontal distances do not have any biological significance.





## Creating a Phylogenetic Tree for Twelve Species

Plotting a simple phylogenetic tree for five species seems to indicate a number of monophyletic groups (see “Creating a Phylogenetic Tree for Five Species” on page 4-6). After a preliminary analysis with five species, you can add more species to your phylogenetic tree. Adding more species to the data set will help you to confirm the observed monophyletic groups are valid.

- 1 Add more sequences to a MATLAB structure. For example, add mtDNA D-loop sequences for other hominid species.

```
data2 = {'Puti_Orangutan'      'AF451972';
        'Jari_Orangutan'     'AF451964';
        'Western_Lowland_Gorilla' 'AY079510';
        'Eastern_Lowland_Gorilla' 'AF050738';
```

```
'Chimp_Schweinfurthii'   'AF176722';  
'Chimp_Vellerosus'     'AF315498';  
'Chimp_Verus'          'AF176731';  
};
```

- 2** Get additional sequence data from the GenBank database, and copy the data into the next indices of a MATLAB structure.

```
for ind = 1:7  
    seqs(ind+5).Header = data2{ind,1};  
    seqs(ind+5).Sequence = getgenbank(data2{ind,2},...  
                                     'sequenceonly', true);  
end
```

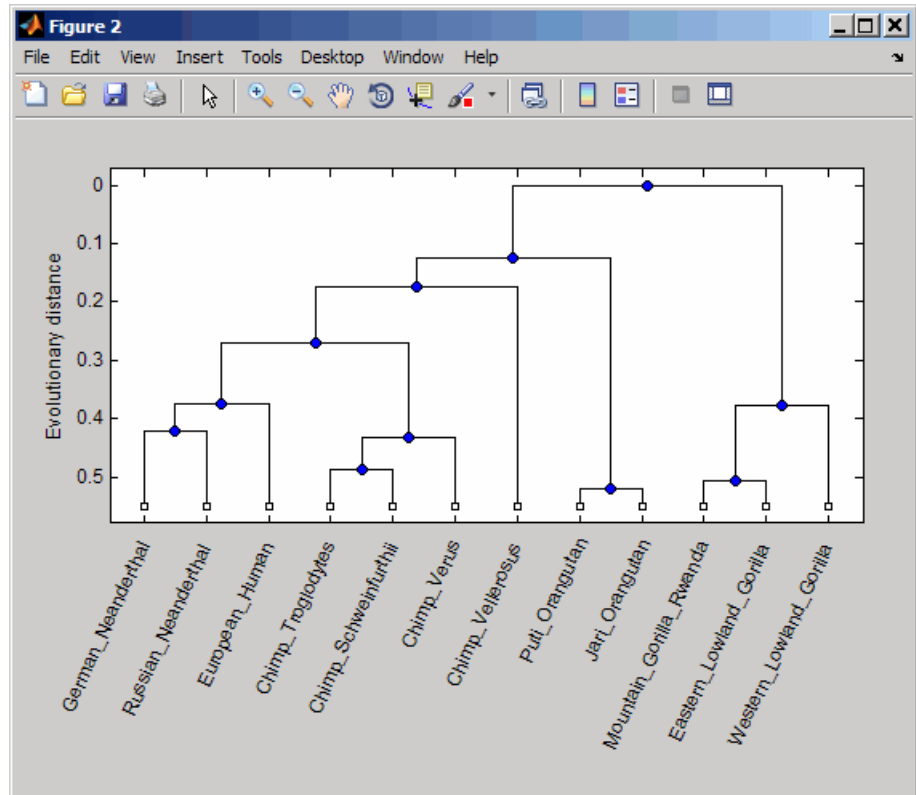
- 3** Calculate pairwise distances and the hierarchical linkage.

```
distances = seqpdist(seqs, 'Method', 'Jukes-Cantor', 'Alpha', 'DNA');  
tree = seqlinkage(distances, 'UPGMA', seqs);
```

- 4** Draw a phylogenetic tree.

```
h = plot(tree, 'orient', 'top');  
ylabel('Evolutionary distance')  
set(h.terminalNodeLabels, 'Rotation', 65)
```

The MATLAB software draws a phylogenetic tree in a Figure window. You can see four main clades for humans, gorillas, chimpanzee, and orangutans.



## Exploring the Phylogenetic Tree

After you create a phylogenetic tree, you can explore the tree using the MATLAB command line or the `phytreeool` GUI. This procedure uses the tree created in “Creating a Phylogenetic Tree for Twelve Species” on page 4-9 as an example.

- 1 List the members of a tree.

```
names = get(tree,'LeafNames')
```

From the list, you can determine the indices for its members. For example, the European Human leaf is the third entry.

```
names =
```

```
'German_Neanderthal'  
'Russian_Neanderthal'  
'European_Human'  
'Chimp_Troglodytes'  
'Chimp_Schweinfurthii'  
'Chimp_Verus'  
'Chimp_Vellerosus'  
'Puti_Orangutan'  
'Jari_Orangutan'  
'Mountain_Gorilla_Rwanda'  
'Eastern_Lowland_Gorilla'  
'Western_Lowland_Gorilla'
```

- 2** Find the closest species to a selected species in a tree. For example, find the species closest to the European human.

```
[h_all,h_leaves] = select(tree,'reference',3,...  
                          'criteria','distance',...  
                          'threshold',0.6);
```

`h_all` is a list of indices for the nodes within a patristic distance of 0.6 to the European human leaf, while `h_leaves` is a list of indices for only the leaf nodes within the same patristic distance.

A patristic distance is the path length between species calculated from the hierarchical clustering distances. The path distance is not necessarily the biological distance.

- 3** List the names of the closest species.

```
subtree_names = names(h_leaves)
```

The MATLAB software prints a list of species with a patristic distance to the European human less than the specified distance. In this case, the patristic distance threshold is less than 0.6.

```
subtree_names =  
  
'German_Neanderthal'  
'Russian_Neanderthal'
```

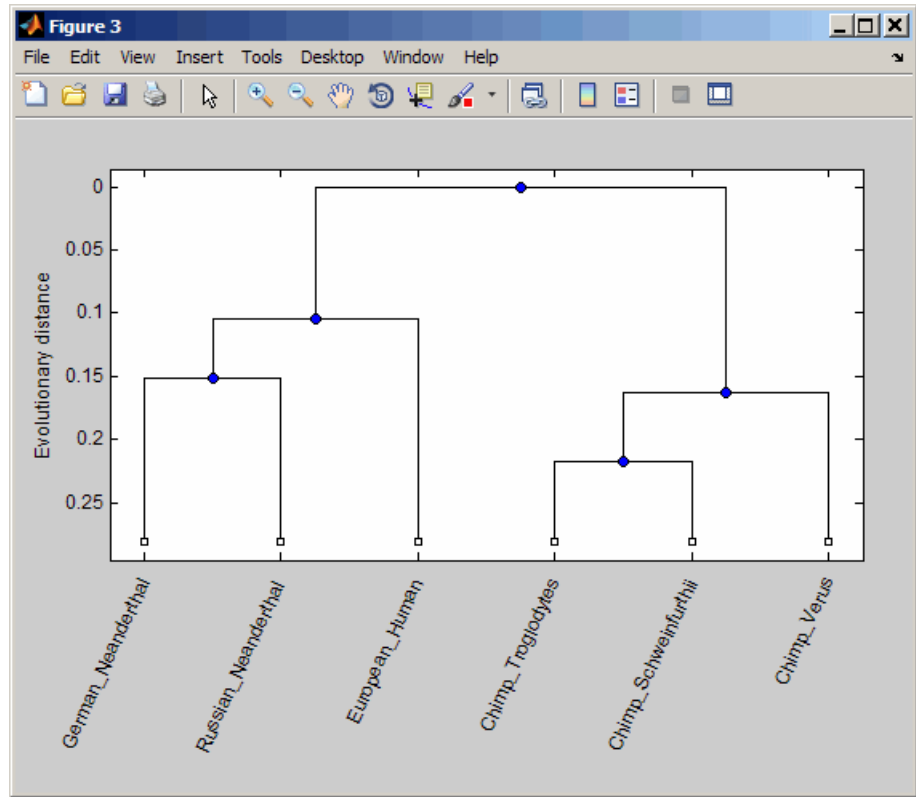
```
'European_Human'  
'Chimp_Schweinfurthii'  
'Chimp_Verus'  
'Chimp_Troglodytes'
```

- 4** Extract a subtree from the whole tree by removing unwanted leaves. For example, prune the tree to species within 0.6 of the European human species.

```
leaves_to_prune = ~h_leaves;  
pruned_tree = prune(tree,leaves_to_prune)  
h = plot(pruned_tree,'orient','top');  
ylabel('Evolutionary distance')  
set(h.terminalNodeLabels,'Rotation',65)
```

The MATLAB software returns information about the new subtree and plots the pruned phylogenetic tree in a Figure window.

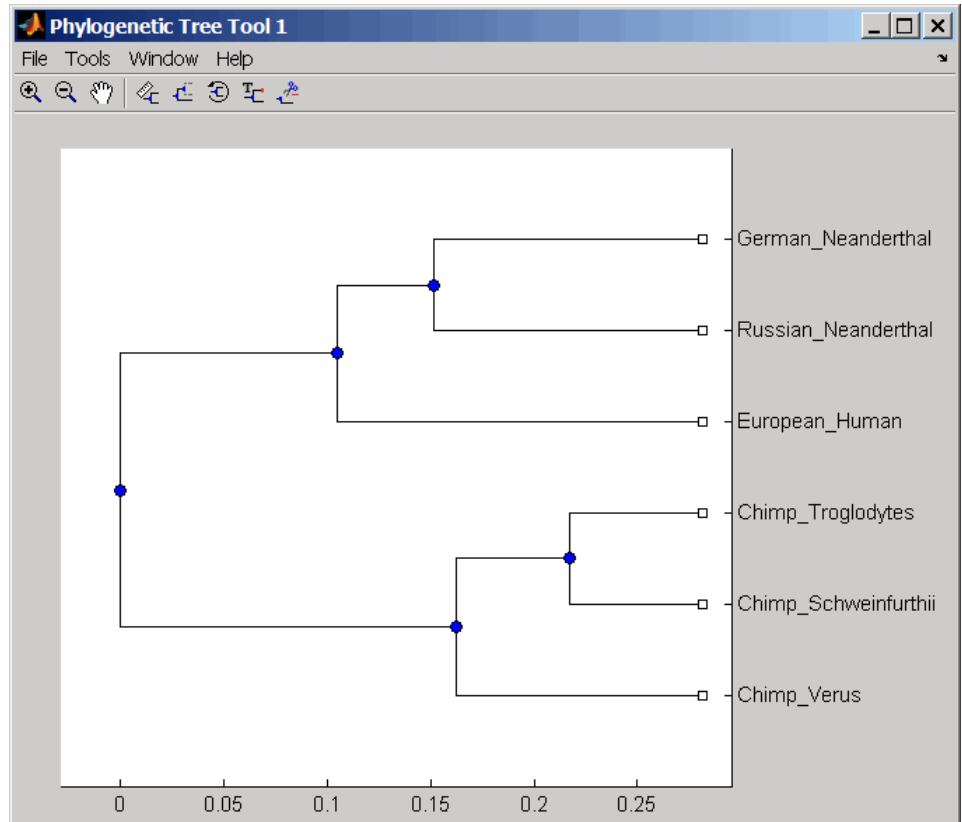
```
Phylogenetic tree object with 6 leaves (5 branches)
```



- 5 Explore, edit, and format a phylogenetic tree using an interactive GUI.

```
phytreetool(pruned_tree)
```

The Phylogenetic Tree Tool window opens and draws the tree.



You can interactively change the appearance of the tree within the tool window. For information on using this GUI, see “Phylogenetic Tree Tool Reference” on page 4-16.

## Phylogenetic Tree Tool Reference

### In this section...

“Overview of the Phylogenetic Tree Tool” on page 4-16

“Opening the Phylogenetic Tree Tool” on page 4-16

“File Menu” on page 4-18

“Tools Menu” on page 4-31

“Window Menu” on page 4-40

“Help Menu” on page 4-40

### Overview of the Phylogenetic Tree Tool

The Phylogenetic Tree Tool is an interactive graphical user interface (GUI) that allows you to view, edit, format, and explore phylogenetic tree data. With this GUI you can prune, reorder, rename branches, and explore distances. You can also open or save Newick-formatted files. The following sections give a description of menu commands and features for creating publishable tree figures.

### Opening the Phylogenetic Tree Tool

This section illustrates how to draw a phylogenetic tree from data in a `phytree` object or a previously saved file.

The Phylogenetic Tree Tool can read data from Newick and ClustalW tree formatted files.

This procedure uses the phylogenetic tree data stored in the file `pf00002.tree` as an example. The data was retrieved from the protein family (PFAM) Web database and saved to a file using the accession number PF00002 and the function `gethmmtree`.

- 1 Create a `phytree` object. For example, to create a `phytree` object from tree data in the file `pf00002.tree`, type

```
tr= phytreeread('pf00002.tree')
```



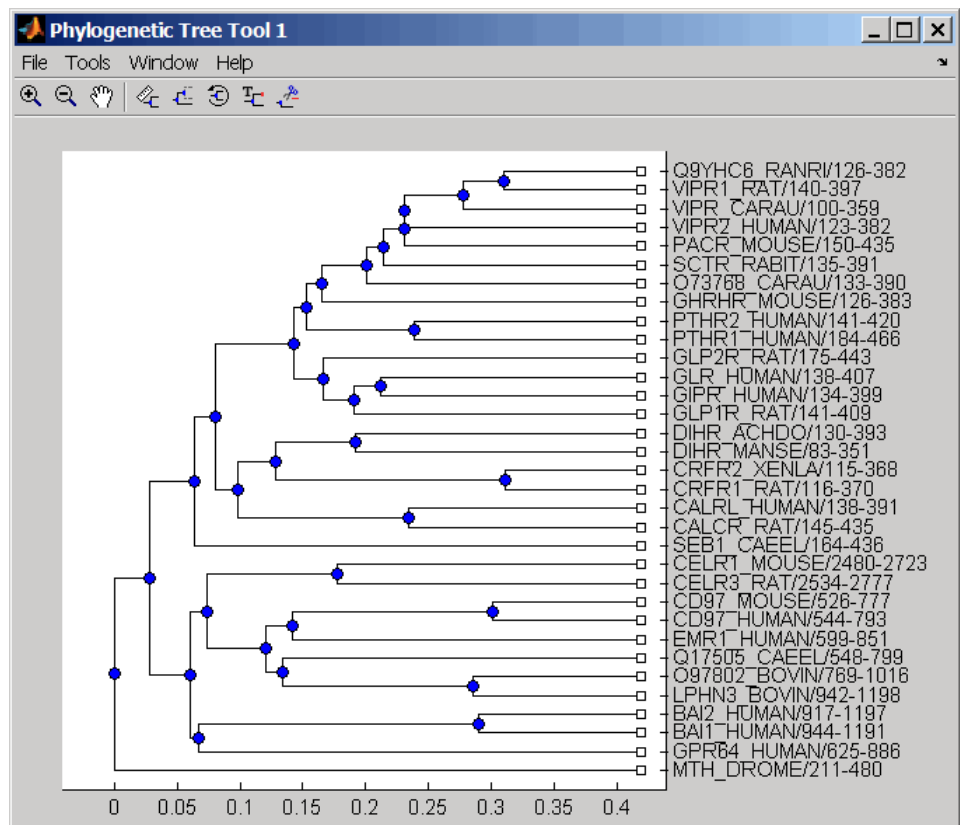
The MATLAB software creates a phytree object.

Phylogenetic tree object with 33 leaves (32 branches)

**2** Open the Phylogenetic Tree Tool and draw a phylogenetic tree.

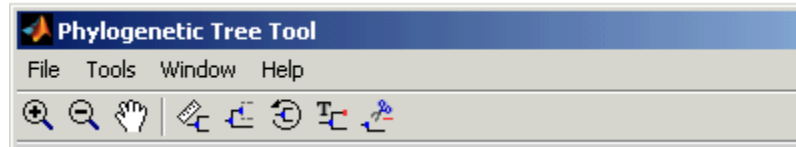
```
phytreetool(tr)
```

The Phylogenetic Tree Tool window opens.



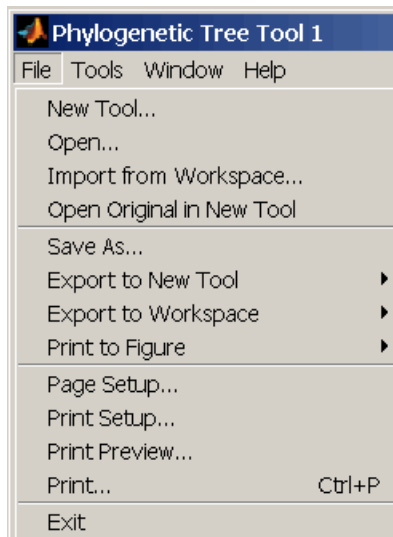
Alternately, if you do not give the `phytreetool` function an argument, the Select Phylogenetic Tree dialog box opens. Select a Newick-formatted file and then click **Open**.

3 Select a command from the menu or toolbar.



### File Menu

The **File** menu includes the standard commands for opening and closing a file, and it includes commands to use phytree object data from the MATLAB Workspace. The **File** menu commands are shown below.

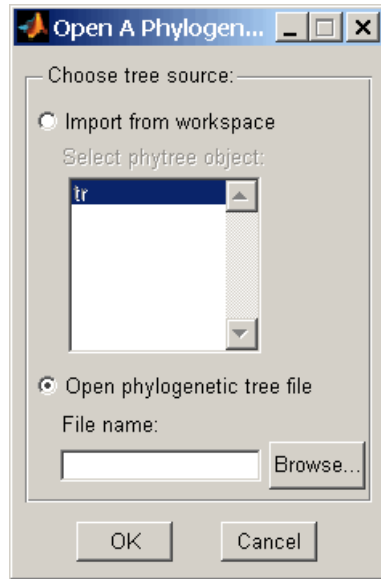


### New Tool Command

Use the **New Tool** command to open tree data from a file into a second Phylogenetic Tree Tool window.

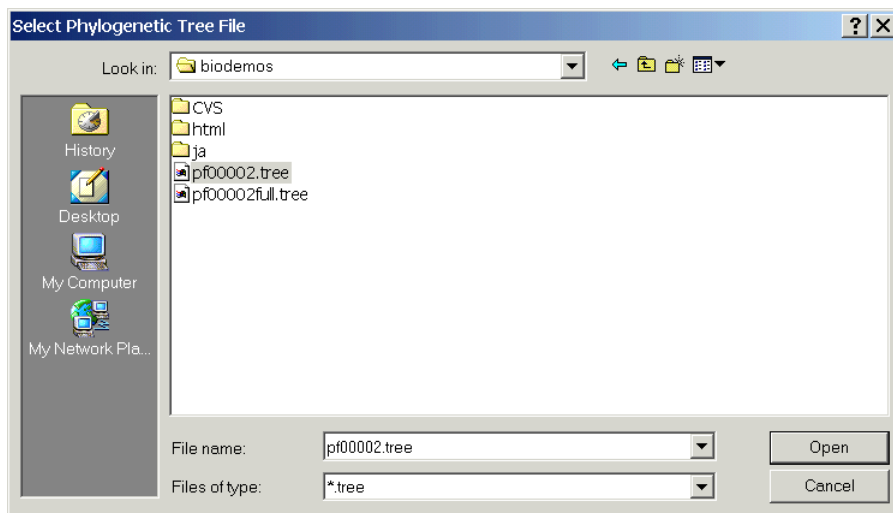
1 From the **File** menu, select **New Tool**.

The Open A Phylogenetic Tree dialog box opens.



## 2 Choose the source for a tree.

- **MATLAB Workspace** — Select the **Import from Workspace** options, and then select a phytree object from the list.
- **File** — Select the **Open phylogenetic tree file** option, click the **Browse** button, select a directory, select a file with the extension `.tree`, and then click **Open**. The toolbox uses the file extension `.tree` for Newick-formatted files, but you can use any Newick-formatted file with any extension.



A second Phylogenetic Tree Tool window opens with tree data from the selected file.

### Open Command

Use the **Open** command to read tree data from a Newick-formatted file and display that data in a Phylogenetic Tree Tool.

- 1 From the **File** menu, click **Open**.

The Select Phylogenetic Tree File dialog box opens.

- 2 Select a directory, select a Newick-formatted file, and then click **Open**. The toolbox uses the file extension `.tree` for Newick-formatted files, but you can use any Newick-formatted file with any extension.

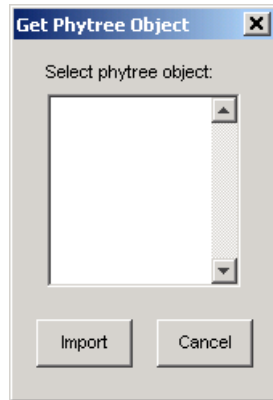
The MATLAB software replaces the current tree data with data from the selected file.

### Import from Workspace Command

Use the **Import from Workspace** command to read tree data from a `phytree` object in the MATLAB Workspace and display that data in a Phylogenetic Tree Tool.

- 1 From the **File** menu, select **Import from Workspace**.

The Get Phytree Object dialog box opens.



- 2 From the list, select a phytree object in the MATLAB Workspace.
- 3 Click the **Import** button.

The MATLAB software replaces the current tree data in the Phylogenetic Tree Tool with data from the selected object.

### Open Original in New Tool

There may be times when you make changes that you would like to undo. Phytreetool does not have an undo command, but you can get back to the original tree you started viewing with the **Open Original in New Tool** command.

From the **File** menu, select **Open Original in New Tool**.

A new Phylogenetic Tree Tool window opens with the original tree.

### Save As Command

After you create a phytree object or prune a tree from existing data, you can save the resulting tree in a Newick-formatted file. The sequence data used to create the phytree object is not saved with the tree.

- 1 From the **File** menu, select **Save As**.

The Save Phylogenetic tree as dialog box opens.

- 2 In the **Filename** box, enter the name of a file. The toolbox uses the file extension `.tree` for Newick-formatted files, but you can use any file extension.
- 3 Click **Save**.

phytreetool saves tree data without the deleted branches, and it saves changes to branch and leaf names. Formatting changes such as branch rotations, collapsed branches, and zoom settings are not saved in the file.

### **Export to New Tool Command**

Because some of the Phylogenetic Tree Tool commands cannot be undone (for example, the Prune command), you might want to make a copy of your tree before trying a command. At other times, you might want to compare two views of the same tree, and copying a tree to a new tool window allows you to make changes to both tree views independently .

- 1 Select **File > Export to New Tool**, and then select either **With Hidden Nodes** or **Only Displayed**.

A new Phylogenetic Tree Tool window opens with a copy of the tree.

- 2 Use the new figure to continue your analysis.

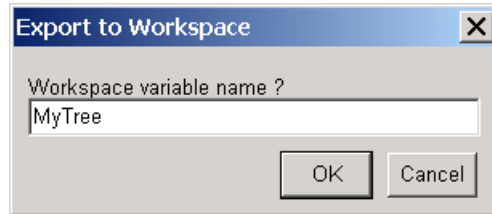
### **Export to Workspace Command**

The Phylogenetic Tree Tool can open Newick-formatted files with tree data. However, it does not create a phytree object in the MATLAB Workspace. If you want to programmatically explore phylogenetic trees, you need to use the **Export to Workspace** command.

- 1 Select **File > Export to Workspace**, and then select either **With Hidden Nodes** or **Only Displayed**.

The Export to Workspace dialog box opens.

- 2** In the **Workspace variable name** box, enter the name for your phylogenetic tree data. For example, enter MyTree.



- 3** Click **OK**.

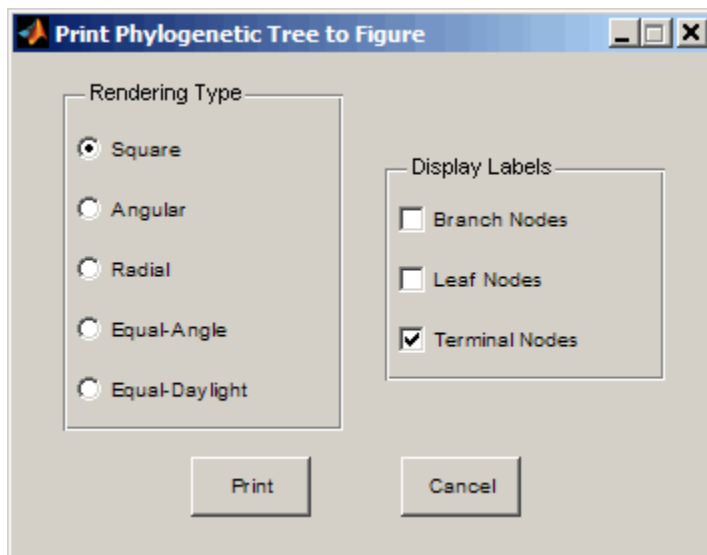
The `phytreetool` creates a `phytree` object in the MATLAB Workspace.

### **Print to Figure Command**

After you have explored the relationships between branches and leaves in your tree, you can copy the tree to a MATLAB Figure window. Using a Figure window lets you use all the features for annotating, changing font characteristics, and getting your figure ready for publication. Also, from the Figure window, you can save an image of the tree as it was displayed in the Phylogenetic Tree Tool window.

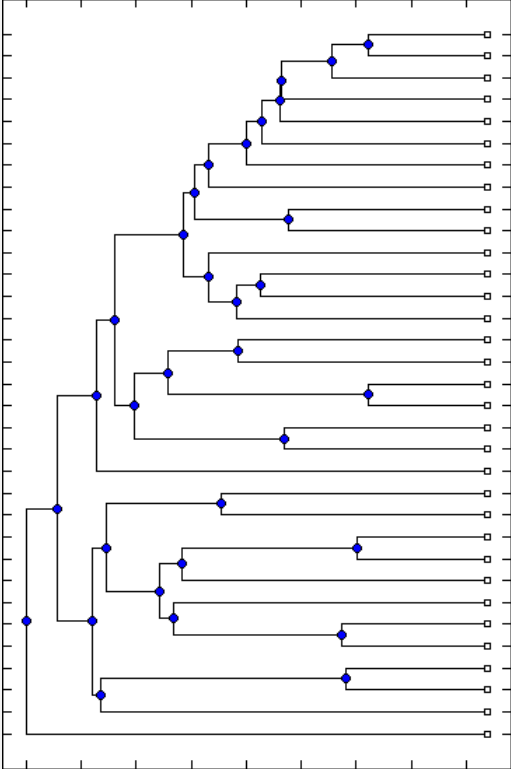
- 1** From the **File** menu, select **Print to Figure**, and then select either **With Hidden Nodes** or **Only Displayed**.

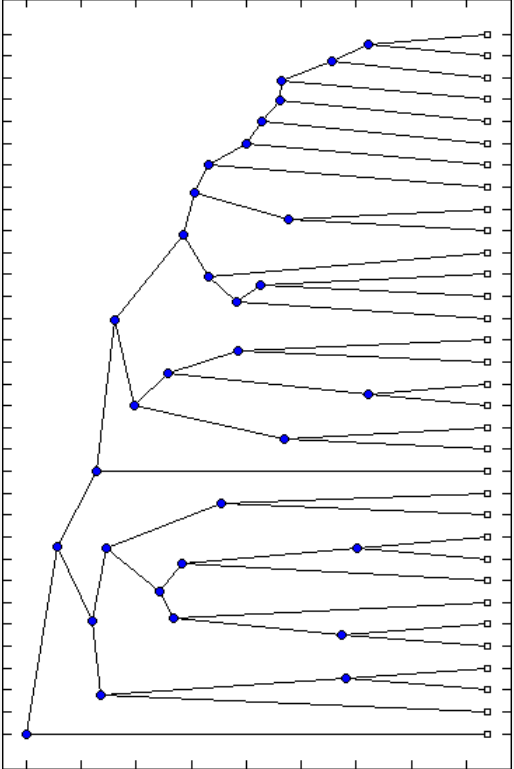
The Publish Phylogenetic Tree to Figure dialog box opens.

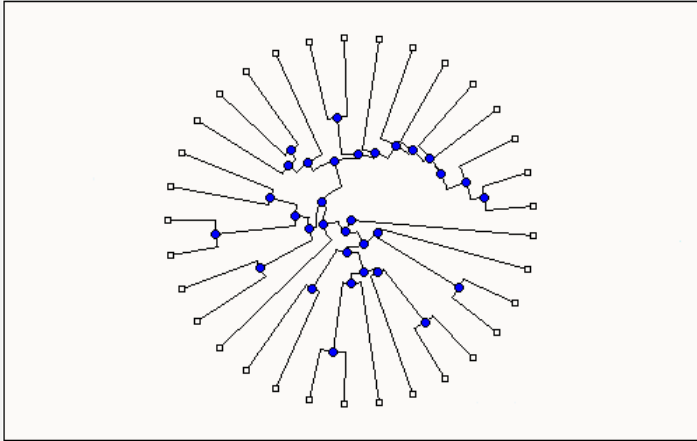
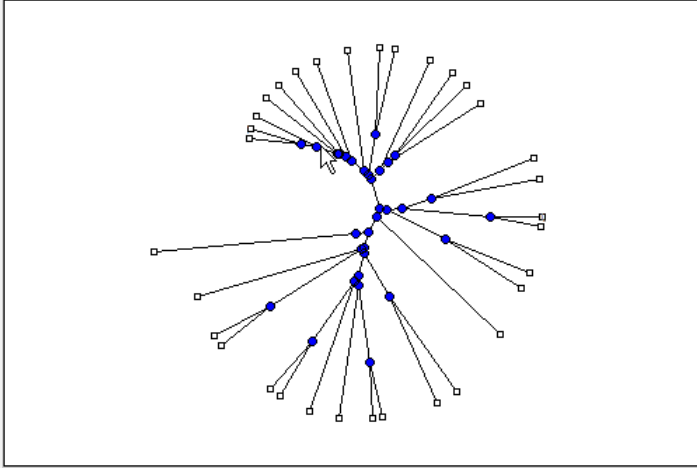


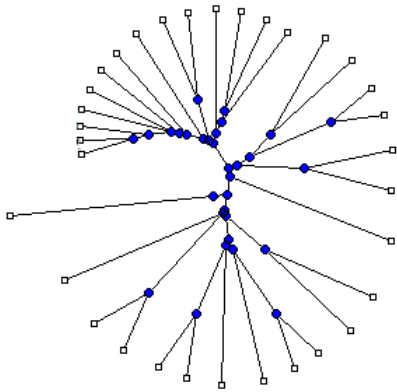
2 Select one of the **Rendering Types**.



Rendering Type	Description
'square' (default)	

Rendering Type	Description
'angular'	

Rendering Type	Description
'radial'	
'equalangle'	

Rendering Type	Description
	<p><b>Tip</b> This rendering type hides the significance of the root node and emphasizes clusters, thereby making it useful for visually assessing clusters and detecting outliers.</p>
'equaldaylight'	<div data-bbox="713 604 1415 1065" style="border: 1px solid black; padding: 10px; text-align: center;">  </div> <p><b>Tip</b> This rendering type hides the significance of the root node and emphasizes clusters, thereby making it useful for visually assessing clusters and detecting outliers.</p>

- 3** Select the **Display Labels** you want on your figure. You can select from all to none of the options.
- **Branch Nodes** — Display branch node names on the figure.
  - **Leaf Nodes** — Display leaf node names on the figure.
  - **Terminal Nodes** — Display terminal node names on the right border.

**4** Click the **Print** button.

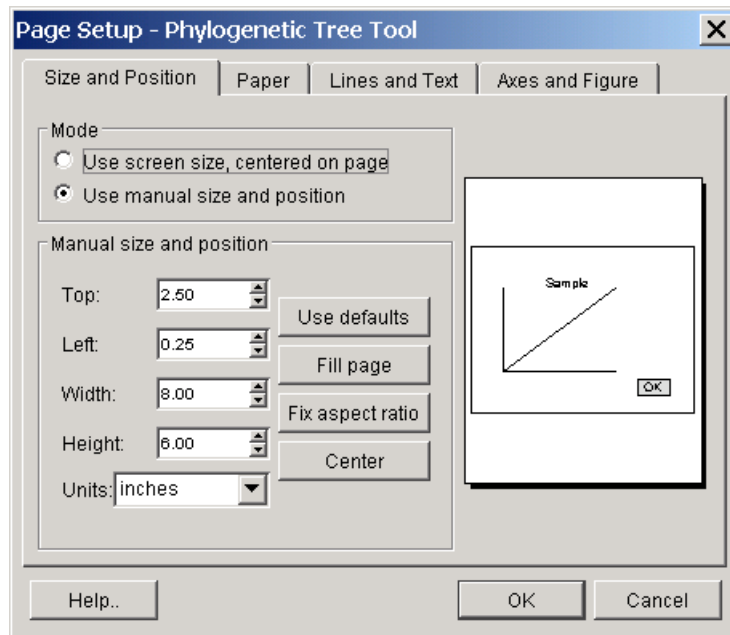
A new Figure window opens with the characteristics you selected.

## Page Setup Command

When you print from the Phylogenetic Tree Tool or a MATLAB Figure window (with a tree published from the tool), you can specify setup options for printing a tree.

**1** From the **File** menu, select **Page Setup**.

The Page Setup - Phylogenetic Tree Tool dialog box opens, which you can use to select page formatting options.



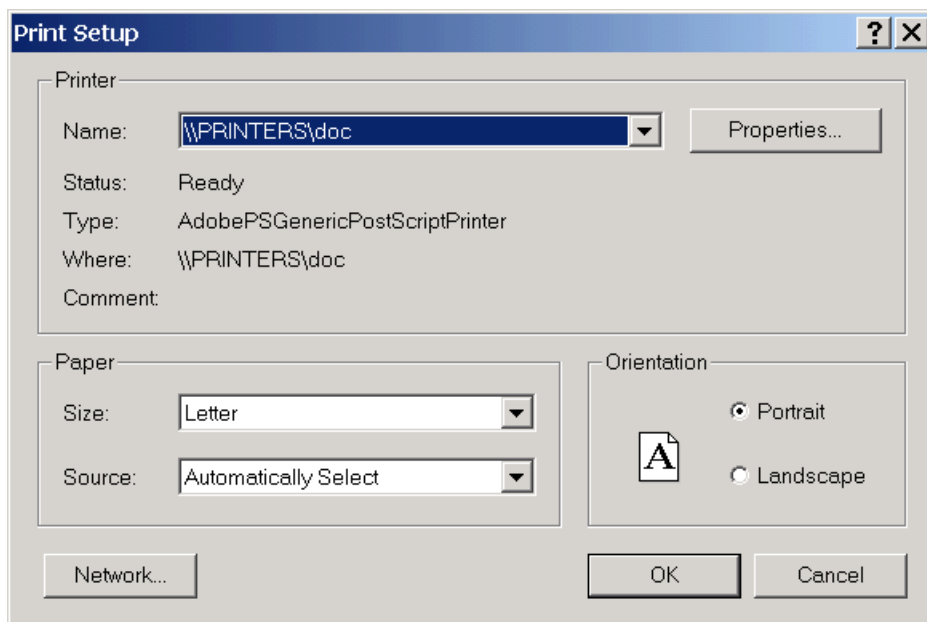
**2** Select the page formatting options and values you want, and then click **OK**.

### Print Setup Command

Use the Print Setup command with the Page Setup command to print a MATLAB Figure window.

- 1 From the **File** menu, select **Print Setup**.

The Print Setup dialog box opens.



- 2 Select the printer and options you want, and then click **OK**.

### Print Preview Command

Use the **Print Preview** command to check the formatting options you selected with the **Page Setup** command.

- 1 From the **File** menu, select **Print Preview**.

A window opens with a picture of your figure with the selected formatting options.

- 2 Click **Print** or **Close**.

### **Print Command**

Use the **Print** command to make a copy of your phylogenetic tree after you use the **Page Setup** command to select formatting options.

- 1 From the **File** menu, select **Print**.

The Print dialog box opens.

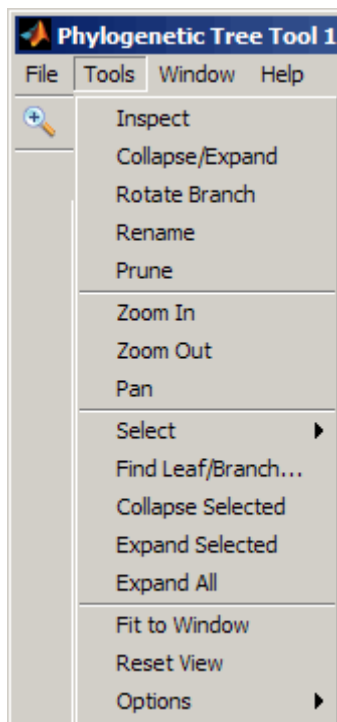
- 2 From the **Name** list, select a printer, and then click **OK**.

### **Tools Menu**

Use the **Tools** menu to:


- Explore branch paths
- Rotate branches
- Find, rename, hide, and prune branches and leaves.

The **Tools** menu and toolbar contain most of the commands specific to trees and phylogenetic analysis. Use these commands and modes to edit and format your tree interactively. The **Tools** menu commands are:



### Inspect Mode

Viewing a phylogenetic tree in the Phylogenetic Tree Tool window provides a rough idea of how closely related two sequences are. However, to see exactly how closely related two sequences are, measure the distance of the path between them. Use the **Inspect** command to display and measure the path between two sequences.

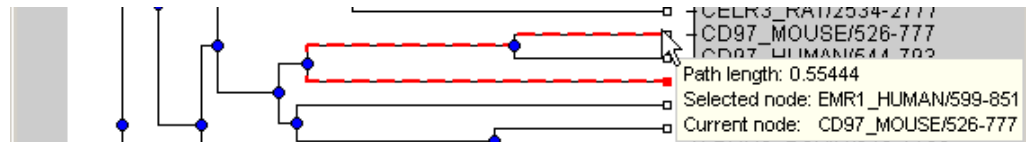
- 1 Select **Tools > Inspect**, or from the toolbar, click the Inspect Tool Mode icon .

The Phylogenetic Tree Tool is set to inspect mode.

- 2 Click a branch or leaf node (selected node), and then hover your cursor over another branch or leaf node (current node).




The tool highlights the path between the two nodes and displays the path length in the pop-up window. The path length is the patristic distance calculated by the `seqpdist` function.



### Collapse and Expand Branch Mode

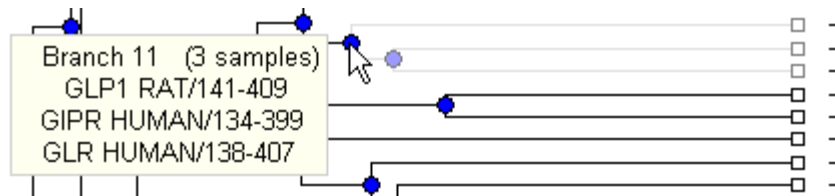
Some trees have thousands of leaf and branch nodes. Displaying all the nodes can create an unreadable tree diagram. By collapsing some branches, you can better see the relationships between the remaining nodes.

- 1 Select **Tools > Collapse/Expand**, or from the toolbar, click the Collapse/Expand Branch Mode icon .

The Phylogenetic Tree Tool is set to collapse/expand mode.

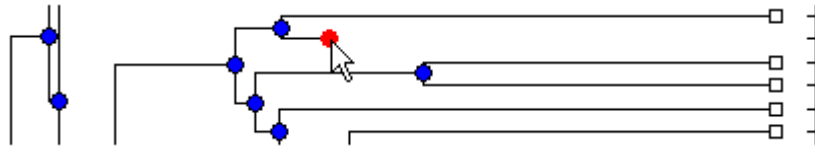
- 2 Point to a branch.

The paths, branch nodes, and leaf nodes below the selected branch appear in gray, indicating you selected them to collapse (hide from view).



- 3 Click the branch node.

The tool hides the display of paths, branch nodes, and leaf nodes below the selected branch. However, it does not remove the data.



**4** To expand a collapsed branch, click it or select **Tools > Reset View**.


---

**Tip** After collapsing nodes, you can redraw the tree by selecting **Tools > Fit to Window**.

---

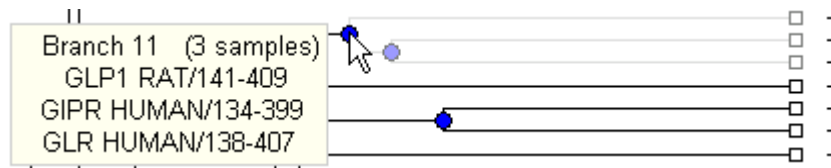
### Rotate Branch Mode

A phylogenetic tree is initially created by pairing the two most similar sequences and then adding the remaining sequences in a decreasing order of similarity. You can rotate branches to emphasize the direction of evolution.

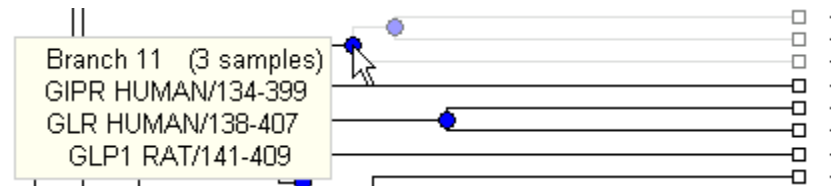
**1** Select **Tools > Rotate Branch**, or from the toolbar, click the Rotate Branch Mode icon .

The Phylogenetic Tree Tool is set to rotate branch mode.

**2** Point to a branch node.



**3** Click the branch node.




The branch and leaf nodes below the selected branch node rotate 180 degrees around the branch node.

- 4 To undo the rotation, simply click the branch node again.

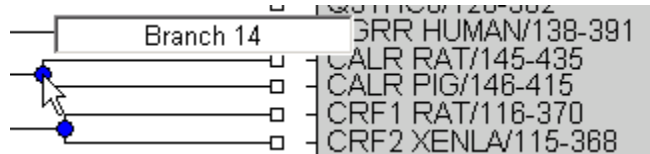
### Rename Leaf or Branch Mode

The Phylogenetic Tree Tool takes the node names from the phytree object and creates numbered branch names starting with Branch 1. You can edit any of the leaf or branch names.

- 1 Select **Tools > Rename**, or from the toolbar, click the Rename Leaf/Branch Mode icon .

The Phylogenetic Tree Tool is set to rename mode.

- 2 Click a branch or leaf node.



A text box opens with the current name of the node.


- 3 In the text box, edit or enter a new name.



- 4 To accept your changes and close the text box, click outside of the text box. To save your changes, select **File > Save As**.

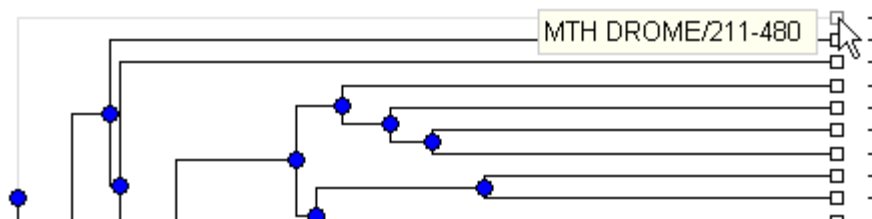
### Prune (Delete) Leaf or Branch Mode

Your tree can contain leaves that are far outside the phylogeny, or it can have duplicate leaves that you want to remove.

- 1 Select **Tools > Prune**, or from the toolbar, click the Prune (delete) Leaf/Branch Mode icon .

The Phylogenetic Tree Tool is set to prune mode.

- 2 Point to a branch or leaf node.



For a leaf node, the branch line connected to the leaf appears in gray. For a branch node, the branch lines below the node appear in gray.

---

**Note** If you delete nodes (branches or leaves), you cannot undo the changes. The Phylogenetic Tree Tool does not have an Undo command.

---

- 3 Click the branch or leaf node.

The tool removes the branch from the figure and rearranges the other nodes to balance the tree structure. It does not recalculate the phylogeny.


---

**Tip** After pruning nodes, you can redraw the tree by selecting **Tools > Fit to Window**.

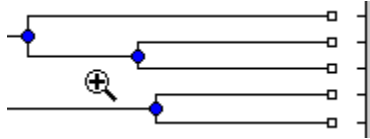
---

### Zoom In, Zoom Out, and Pan Commands

The Zoom and Pan commands are the standard controls for resizing and moving the screen in any MATLAB Figure window.

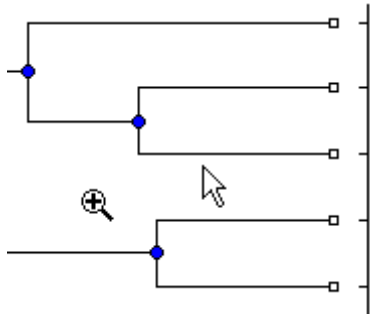
- 1 Select **Tools > Zoom In**, or from the toolbar, click the Zoom In icon .


The tool activates zoom in mode and changes the cursor to a magnifying glass.



- 2 Place the cursor over the section of the tree diagram you want to enlarge and then click.

The tree diagram doubles its size.



- 3 From the toolbar click the Pan icon .
- 4 Move the cursor over the tree diagram, left-click, and drag the diagram to the location you want to view.

---

**Tip** After zooming and panning, you can reset the tree to its original view, by selecting **Tools > Reset View**.

---

### Select Submenu

Select a single branch or leaf node by clicking it. Select multiple branch or leaf nodes by **Shift**-clicking the nodes, or click-dragging to draw a box around nodes.

Use the **Select** submenu to select specific branch and leaf nodes based on different criteria.

- **Select By Distance** — Displays a slider bar at the top of the window, which you slide to specify a distance threshold. Nodes whose distance from the selected node are below this threshold appear in red. Nodes whose distance from the selected node are above this threshold appear in blue.
- **Select Common Ancestor** — For all selected nodes, highlights the closest common ancestor branch node in red.
- **Select Leaves** — If one or more nodes are selected, highlights the nodes that are leaf nodes in red. If no nodes are selected, highlights all leaf nodes in red
- **Propagate Selection** — For all selected nodes, highlights the descendant nodes in red.
- **Swap Selection** — Clears all selected nodes and selects all deselected nodes.

After selecting nodes using one of the previous commands, hide and show the nodes using the following commands:

- **Collapse Selected**
- **Expand Selected**
- **Expand All**

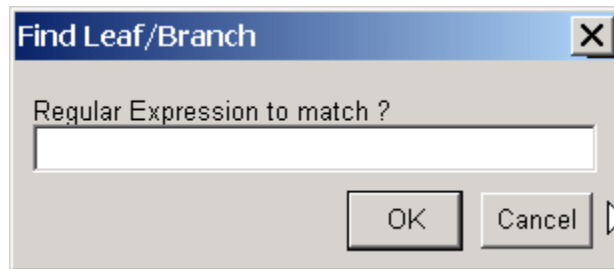
Clear all selected nodes by clicking anywhere else in the Phylogenetic Tree Tool window.

### **Find Leaf or Branch Command**

Phylogenetic trees can have thousands of leaves and branches, and finding a specific node can be difficult. Use the **Find Leaf/Branch** command to locate a node using its name or part of its name.

#### **1 Select Tools > Find Leaf/Branch.**

The Find Leaf/Branch dialog box opens.



- 2** In the **Regular Expression to match** box, enter a name or partial name of a branch or leaf node.
- 3** Click **OK**.

The branch or leaf nodes that match the expression appear in red.

After selecting nodes using the **Find Leaf/Branch** command, you can hide and show the nodes using the following commands:

- **Collapse Selected**
- **Expand Selected**
- **Expand All**

### **Collapse Selected, Expand Selected, and Expand All Commands**

When you select nodes, either manually or using the previous commands, you can then collapse them by selecting **Tools > Collapse Selected**.

The data for branches and leaves that you hide using the **Collapse/Expand** or **Collapse Selected** command are not removed from the tree. You can display selected or all hidden data using the **Expand Selected** or **Expand All** command.

### **Fit to Window Command**

After you hide nodes with the collapse commands, or delete nodes with the **Prune** command, there can be extra space in the tree diagram. Use the **Fit**

to **Window** command to redraw the tree diagram to fill the entire Figure window.

Select **Tools > Fit to Window**.

### **Reset View Command**

Use the **Reset View** command to remove formatting changes such as collapsed branches and zooms.

Select **Tools > Reset View**.

### **Options Submenu**

Use the **Options** command to select the behavior for the zoom and pan modes.

- **Unconstrained Zoom** — Allow zooming in both horizontal and vertical directions.
- **Horizontal Zoom** — Restrict zooming to the horizontal direction.
- **Vertical Zoom** (default) — Restrict zooming to the vertical direction.
- **Unconstrained Pan** — Allow panning in both horizontal and vertical directions.
- **Horizontal Pan** — Restrict panning to the horizontal direction.
- **Vertical Pan** (default) — Restrict panning to the vertical direction.

### **Window Menu**

This section illustrates how to switch to any open window.

The **Window** menu is standard on MATLAB interfaces and Figure windows. Use this menu to select any opened window.

### **Help Menu**

This section illustrates how to select quick links to the Bioinformatics Toolbox documentation for phylogenetic analysis functions, tutorials, and the `phytreetool` reference



Use the **Help** menu to select quick links to the Bioinformatics Toolbox documentation for phylogenetic analysis functions, tutorials, and the `phytreetool` reference.



# Examples

---

Use this list to find examples in the documentation.

## **Introduction**

“Importing and Exploring Bioinformatic Data from Microsoft® Excel” on page 1-21

“Creating get Functions” on page 1-29

“Extracting Information from Large Multi-Entry Text Files” on page 1-34

## **Sequence Analysis**

“Example: Sequence Statistics” on page 2-2

“Example: Sequence Alignment” on page 2-22

“Storing and Managing Short-Read Sequence Data in Objects” on page 2-63

## **Microarray Analysis**

“Storing and Managing Gene Expression Data in Objects” on page 3-2

“Representing Expression Data Values in DataMatrix Objects” on page 3-5

“Representing Expression Data Values in ExptData Objects” on page 3-11

“Representing Sample and Feature Metadata in MetaData Objects” on page 3-15

“Representing Experiment Information in a MIAME Object” on page 3-22

“Example: Visualizing Microarray Data” on page 3-33

“Example: Analyzing Gene Expression Profiles” on page 3-57

## **Phylogenetic Analysis**

“Example: Building a Phylogenetic Tree” on page 4-3

## A

- amino acids
  - comparing sequences 2-33
  - composition 2-18
- applications
  - deploying 1-19
  - prototyping 1-19

## B

- bioinformatics
  - application deployment 1-20
  - computation with MATLAB 1-2
  - data visualization 1-19
  - visualizing data 1-2
- Bioinformatics Toolbox
  - additional software 1-6
  - expected user 1-3
  - installation 1-5
  - required software 1-5

## C

- clusters
  - gene expression data 3-64
- codons
  - nucleotide composition 2-11
- composition
  - amino acid 2-18
  - nucleotide 2-11
- conversions
  - nucleotide to amino acid 2-18

## D

- data
  - filtering microarray data 3-61
  - getting into MATLAB 2-5
  - loading into MATLAB 3-57
  - microarray 3-34

- data formats
  - supporting functions 1-8
- data visualization
  - bioinformatics 1-19
- databases
  - getting information from 2-24
  - related genes 2-27
  - supporting functions 1-8
- DataMatrix object
  - accessing 3-8
  - constructing 3-6
  - getting properties 3-7
  - indexing 3-8
  - properties 3-7
  - setting properties 3-7
  - working with 3-5

## E

- examples
  - gene expression in mouse brain 3-33
  - gene expression in yeast metabolism 3-57
  - sequence alignment 2-22
  - sequence statistics 2-2
- Excel spreadsheets
  - using with bioinformatics data 1-21

## F

- FASTA files
  - accessing 1-34
- FASTQ files
  - accessing 1-34
- features
  - prototyping 1-19
- functions
  - data formats 1-8
  - databases 1-8
  - graph theory 1-17
  - mass spectrometry analysis 1-14

- microarray analysis 1-12
- microarray data storage 1-13 3-5
- protein structure analysis 1-11
- sequence alignment 1-10
- sequence utilities 1-10
- statistical learning 1-18

## **G**

- gene expression profile
  - mouse brain 3-33
  - yeast metabolism 3-57
- genome data
  - with MATLAB structures 3-57
- graph theory
  - supporting functions 1-17
- graph visualization
  - supporting methods 1-18

## **I**

- installation
  - from DVD or Web 1-5

## **M**

- mass spectrometry analysis
  - supporting functions 1-14
- MATLAB structures
  - with genome data 2-5
- methods
  - graph visualization 1-18
- microarray
  - clustering genes 3-64
  - filtering data 3-61
  - mouse brain example 3-1
  - principal component analysis 3-68
  - scatter plots 3-48
  - spacial images 3-36
  - statistics 3-46
  - visualizing data 3-33

- working with data 3-34
  - yeast example 3-1

- microarray analysis
  - supporting functions 1-12
- microarray data storage
  - supporting functions 1-13 3-5
- model organism
  - finding 2-22
- mouse brain
  - gene expression profile 3-33
  - microarray tutorial 3-33
- multiple sequence alignment
  - aligning sequences 2-58
  - manual adjustment 2-59
- Multiple Sequence Alignment Viewer
  - GUI 2-56

## **N**

- NCBI
  - searching Web site 2-22
- Next generation sequencing 2-63
- nucleotides
  - composition in sequences 2-6
  - content in sequences 2-2
  - searching database 2-27

## **O**

- objects
  - DataMatrix 3-5
- open reading frames
  - searching for 2-15

## **P**

- phylogenetic analysis
  - building tree 4-3
  - creating subtree 4-9
  - creating tree 4-6
  - exploring tree 4-11

- GUI reference 4-16
- reading data 2-56
- searching NCBI 4-5
- selecting subtree 2-57
- plots
  - scatter 3-48
- principal component analysis
  - filtering microarray data 3-68
- protein properties
  - analysis functions 1-11
- protein sequence
  - locating 2-29
- prototyping
  - supporting features 1-19

## **S**

- SAM files
  - accessing 1-34
- sequence
  - amino acid conversion 2-18
  - codon composition 2-11
  - comparing amino acids 2-33
  - nucleotide content 2-2
  - protein coding 2-29
  - searching database 2-27
  - statistics example 2-2
- sequence alignment
  - example 2-22
  - supporting functions 1-10
- sequence analysis
  - defined 2-1
  - using seqtool GUI 2-42

- Sequence data
  - short-read 2-63
- sequence tool GUI
  - importing sequence 2-42
  - reading frames 2-48
  - searching words 2-46
  - statistics 2-51
  - viewing sequence 2-44
- sequence utilities
  - supporting functions 1-10
- sequences
  - nucleotide composition 2-6
- share algorithms
  - bioinformatics 1-20
- Short-read sequence data 2-63
- software
  - required 1-5
- spatial images
  - microarray 3-36
- Spreadsheet Link EX software
  - using with bioinformatics data 1-21
- statistical learning
  - supporting functions 1-18
- statistics
  - microarray 3-46
- structures
  - with genome data 3-57

## **V**

- visualizing data
  - microarray 3-33