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Bioinformatics Toolbox™ User's Guide


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- “Get Information from Web Database” on page 1-36
Bioinformatics Toolbox Product Description
Read, analyze, and visualize genomic and proteomic data

Bioinformatics Toolbox provides algorithms and apps for Next Generation Sequencing (NGS), microarray analysis, mass spectrometry, and gene ontology. Using toolbox functions, you can read genomic and proteomic data from standard file formats such as SAM, FASTA, CEL, and CDF, as well as from online databases such as the NCBI Gene Expression Omnibus and GenBank®. You can explore and visualize this data with sequence browsers, spatial heatmaps, and clustergrams. The toolbox also provides statistical techniques for detecting peaks, imputing values for missing data, and selecting features.

You can combine toolbox functions to support common bioinformatics workflows. You can use ChIP-Seq data to identify transcription factors; analyze RNA-Seq data to identify differentially expressed genes; identify copy number variants and SNPs in microarray data; and classify protein profiles using mass spectrometry data.

Key Features

• Next Generation Sequencing analysis and browser
• Sequence analysis and visualization, including pairwise and multiple sequence alignment and peak detection
• Microarray data analysis, including reading, filtering, normalizing, and visualization
• Mass spectrometry analysis, including preprocessing, classification, and marker identification
• Phylogenetic tree analysis
• Graph theory functions, including interaction maps, hierarchy plots, and pathways
• Data import from genomic, proteomic, and gene expression files, including SAM, FASTA, CEL, and CDF, and from databases such as NCBI and GenBank
Product Overview

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.
- **High-throughput sequencing** — Gene expression and transcription factor analysis of next-generation sequencing data, including RNA-Seq and ChIP-Seq.
- **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 1-25.

- **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 1-26.

- **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 1-27.

**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

Installing

Install the Bioinformatics Toolbox software from a DVD or Web release using the MathWorks® Installer.

Required Software

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

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<th>Required Software</th>
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<tr>
<td>MATLAB</td>
<td>Provides a command-line interface and integrated software environment for the Bioinformatics Toolbox software. Bioinformatics Toolbox software requires the current version of MATLAB.</td>
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</table>

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

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<tr>
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<tr>
<td>Parallel Computing Toolbox™</td>
<td>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.</td>
</tr>
<tr>
<td>Signal Processing Toolbox™</td>
<td>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.</td>
</tr>
<tr>
<td>Image Processing Toolbox™</td>
<td>Create complex and custom image processing algorithms for data from microarray scanners.</td>
</tr>
<tr>
<td>SimBiology®</td>
<td>Model, simulate, and analyze biochemical systems.</td>
</tr>
<tr>
<td>Optimization Toolbox™</td>
<td>Use nonlinear optimization to predict the secondary structure of proteins and the structure of other biological macromolecules.</td>
</tr>
<tr>
<td>Deep Learning Toolbox™</td>
<td>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.</td>
</tr>
<tr>
<td>Database Toolbox™</td>
<td>Create your own in-house databases for sequence data with custom annotations.</td>
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<tr>
<td>MATLAB Compiler™</td>
<td>Create standalone applications from MATLAB GUI applications, and create dynamic link libraries from MATLAB functions to use with any programming environment.</td>
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<tr>
<td>MATLAB Compiler SDK™</td>
<td>Create COM objects to use with any COM-based programming environment.</td>
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<tr>
<td>MATLAB Compiler SDK</td>
<td>Integrate MATLAB applications into your organization's Java® programs by creating a Java wrapper around the application.</td>
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<tr>
<td>MATLAB Compiler</td>
<td>Create Microsoft® Excel® add-in functions from MATLAB functions to use with Excel spreadsheets.</td>
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<td>Optional Software</td>
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<td>Spreadsheet Link™</td>
<td>Connect Microsoft Excel with the MATLAB Workspace to exchange data and to use MATLAB computational and visualization functions. For more information, see “Exchange Bioinformatics Data Between Excel and MATLAB” on page 1-28.</td>
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</tbody>
</table>
Data Formats and Databases

The Bioinformatics Toolbox lets you access many of the databases on the web and other online data repositories. It lets you 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). 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® 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® data in GPR and GAL files (gprread, galread), SPOT data (sptread), Affymetrix® GeneChip® data (affyread), and ImaGene® 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.

See Also

More About

- “High-Throughput Sequencing”
- “Microarray Analysis”
- “Sequence Analysis”
- “Structural Analysis”
- “Mass Spectrometry and Bioanalytics”
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 (seqalignviewer) 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).

See Also

More About

- “Sequence Utilities and Statistics” on page 1-11
- “Sequence Analysis”
- “Data Formats and Databases” on page 1-8
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 (seqviewer).

**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 (aaccount, 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).

See Also

**More About**

- “Sequence Alignments” on page 1-10
- “Sequence Analysis”
• “Protein and Amino Acid Sequence Analysis”
• “Data Formats and Databases” on page 1-8
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).

See Also

More About

- “Protein and Amino Acid Sequence Analysis”
- “Structural Analysis”
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. The Bioinformatics Toolbox provides the following data structure and functions for phylogenetic analysis.

**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 (`phytreeviewer` 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 `phytreeviewer` user interface 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`).

**See Also**

**More About**

- “Sequence Utilities and Statistics” on page 1-11
- “Sequence Analysis”
Microarray Data Analysis Tools

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 lowess 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, redgreencmap), box plots (maboxplot), loglog plots (maloglog), and intensity-ratio plots (mairplot). You can also view clustered expression profiles (clustergram, redgreencmap). 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.
See Also

More About

• “Microarray Data Storage” on page 1-17
• “Microarray Analysis”
Microarray Data Storage

The Bioinformatics 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 4-5.

### See Also

### More About

- “Microarray Data Analysis Tools” on page 1-15
- “Microarray Analysis”
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.
See Also

More About

• “Mass Spectrometry and Bioanalytics”
• “Data Formats and Databases” on page 1-8
Graph Theory Functions

Graph theory functions in the Bioinformatics 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`, `graphisspantree`, `graphmaxflow`, `graphminspantree`, `graphpred2path`, `graphshortestpath`, `graphtopoorder`, and `graphtraverse`.

**See Also**

**More About**

• “Graph Visualization” on page 1-23
• “Network Analysis and Visualization”
Graph Visualization

The Bioinformatics 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).

See Also

More About

- “Graph Theory Functions” on page 1-21
- “Network Analysis and Visualization”
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 and Machine Learning Toolbox software (classify, kmeans, fitctree, and fitrtree).

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 and MATLAB Compiler SDK, you can create standalone applications independent of the MATLAB environment.

- **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 Compiler SDK 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 Compiler to create Excel add-in functions, and then use these functions with Excel spreadsheets.

- **Create Java classes** — Use MATLAB Compiler SDK to automatically generate Java classes from algorithms written in the MATLAB programming language. You can run these classes outside the MATLAB environment.
Exchange Bioinformatics Data Between Excel and MATLAB

In this section...

“Using Excel and MATLAB Together” on page 1-28
“About the Example” on page 1-28
“Before Running the Example” on page 1-28
“Running the Example for the Entire Data Set” on page 1-29
“Editing Formulas to Run the Example on a Subset of the Data” on page 1-31
“Using the Spreadsheet Link product to Interact With the Data in MATLAB” on page 1-33

Using Excel and MATLAB Together

If you have bioinformatics data in an Excel (2007 or newer) spreadsheet, use Spreadsheet Link to:

• Connect Excel with the MATLAB Workspace to exchange data
• Use MATLAB and Bioinformatics Toolbox computational and visualization functions

About the Example

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

The Excel 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 was filtered using the steps described in Gene Expression Profile Analysis.

Before Running the Example

1 If not already done, modify your system path to include the MATLAB root folder as described in the Spreadsheet Link documentation.
2 If not already done, enable the Spreadsheet Link Add-In as described in “Add-In Setup” (Spreadsheet Link).

3 Close MATLAB and Excel if they are open.

4 Start Excel. MATLAB and Spreadsheet Link software automatically start.

5 From Excel, open the following file provided with the Bioinformatics Toolbox software:

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

**Note** `matlabroot` is the MATLAB root folder, which is where MATLAB software is installed on your system.

6 In the Excel software, enable macros. Click the **Developer** tab, and 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. If you encounter the "Can't find project or library" error, you might need to update the references in the Visual Basic software. Open Visual Basic by clicking the **Developer** tab and selecting **Visual Basic**. Then select **Tools > References > SpreadsheetLink**. If the **MISSING: exclink2007.xlam** check box is selected, clear it.

**Running the Example for the Entire Data Set**

1 In the provided Excel file, note that columns A through H contain data from DeRisi et al. Also note that cells J5, J6, J7, and J12 contain formulas using Spreadsheet Link functions `MLPutMatrix` and `MLEvalString`.

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

2 Execute the formulas in cells J5, J6, J7, and J12, by selecting the cell, pressing **F2**, and then pressing **Enter**.

Each of the first three cells contains a formula using the Spreadsheet Link function `MLPutMatrix`, which creates a MATLAB variable from the data in the spreadsheet. Cell J12 contains a formula using the Spreadsheet Link function `MLEvalString`, which runs the Bioinformatics Toolbox `clustergram` function using the three variables as input. For more information on adding formulas using Spreadsheet Link functions, see “Create Diagonal Matrix Using Worksheet Cells” (Spreadsheet Link).
3 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 by clicking the Developer tab, and then clicking the Visual Basic button 📖. (If the Developer tab is not on the Excel ribbon, consult Excel Help to display it.)

For more information on creating macros using Visual Basic Editor, see “Create Diagonal Matrix Using VBA Macro” (Spreadsheet Link).

4 Execute 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.

---

**Editing Formulas to Run the Example on a Subset of the Data**

1  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, and then press **F2** to display the formula for editing. Change H617 to H33, and then press **Enter**.

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

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

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

2. 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**, and then press **Enter**.

   b. Select cell J6, press **F2**, and then press **Enter**.

   c. Select cell J7, press **F2**, and then press **Enter**.

   d. Select cell J12, press **F2**, and then press **Enter**.
Using the Spreadsheet Link product to Interact With the Data in MATLAB

Use the MATLAB group on the right side of the Home tab to interact with the data:
For example, create a variable in MATLAB containing a 3-by-7 matrix of the data, plot the data in a Figure window, and then add the plot to your spreadsheet:

1. Click-drag to select cells B5 through H7.

2. From the MATLAB group, select Send data to MATLAB.

3. Type YAGenes for the variable name, and then click OK.

The variable YAGenes is added to the MATLAB Workspace as a 3-by-7 matrix.

4. From the MATLAB group, select Run MATLAB command.

5. Type `plot(YAGenes')` for the command, and 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.

6. Select cell J20, and then click from the MATLAB group, select Get MATLAB figure.
The figure is added to the spreadsheet.
Get Information from Web Database

In this section...

“What Are get Functions?” on page 1-36
“Creating the getpubmed Function” on page 1-37

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.
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-value pairs that let the user of the function limit the search by publication date and limit the number of records returned. Below is the step-by-step guide to create the function from the beginning. To see the completed m-file, type edit _getpubmed_.m.

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:

```matlab
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.

```matlab
% Error checking for required input SEARCHTERM
if(nargin<1)
    error(message('bioinfo:getpubmed:NotEnoughInputArguments'));
end
```

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

```matlab
% 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-value pairs if provided as input.

```matlab
% Parsing the property name/value pairs
numargin = numel(varargin);
for n = 1:2:numargin
    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};
```

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
```

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®), 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)];
```

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];
```
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.

```matlab
text = urlread(searchURL);
```

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.

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

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

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

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.

```matlab
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
```

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 file is located at:

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

**Note** The notation `matlabroot` is the MATLAB root directory, which is the directory where the MATLAB software is installed on your system.
High-Throughput Sequence Analysis

- “Work with Next-Generation Sequencing Data” on page 2-2
- “Manage Sequence Read Data in Objects” on page 2-8
- “Store and Manage Feature Annotations in Objects” on page 2-21
- “Visualize and Investigate Sequence Read Alignments” on page 2-28
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Work with Next-Generation Sequencing Data

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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 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.

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 vector, 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.

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.
Create 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.

   ```matlab
   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.

   ```matlab
   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.

Determine 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:

```matlab
gene2goObj.NumEntries
```

```matlab
ans =
   6476
```
Retrieve Entries from Your Source File

Retrieve entries from your source file using either:

- The index of the entry
- The entry key

Retrieve 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:

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

Retrieve 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:

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

The output `subset_entries` is a character vector 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.

Read 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.

Set the Interpreter Property

Before using the `read` method, make sure the `Interpreter` property of the `BioIndexedFile` object is set appropriately.
### If you constructed a BioIndexedFile object from ...

<table>
<thead>
<tr>
<th>If you constructed a BioIndexedFile object from ...</th>
<th>The Interpreter property ...</th>
</tr>
</thead>
<tbody>
<tr>
<td>A source file with an application-specific format (FASTA, FASTQ, or SAM)</td>
<td>By default is a handle to a function appropriate for that file type and typically does not require you to change it.</td>
</tr>
<tr>
<td>A source file with a table, multi-row table, or flat format</td>
<td>By default is [], 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 character vector of one or more concatenated entries and returns a structure or an array of structures containing the interpreted data.</td>
</tr>
</tbody>
</table>

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.

### Read 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 character vectors and extracts those that start with the characters GO.

```matlab
gene2goObj.Interpreter = @(x) regexp(x,'GO:\d+','match')
```
Read only the entries that have a key of YAT2, and return their GO terms.

```matlab
GO_YAT2_entries = read(gene2goobj, 'YAT2')
GO_YAT2_entries =
'GO:0004092' 'GO:0005737' 'GO:0006066' 'GO:0006066' 'GO:0009437'
```
Manage Sequence Read Data in Objects

In this section...

“Overview” on page 2-8
“Represent Sequence and Quality Data in a BioRead Object” on page 2-9
“Represent Sequence, Quality, and Alignment/Mapping Data in a BioMap Object” on page 2-10
“Retrieve Information from a BioRead or BioMap Object” on page 2-14
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“Determine Coverage of a Reference Sequence” on page 2-17
“Construct Sequence Alignments to a Reference Sequence” on page 2-18
“Filter Read Sequences Using SAM Flags” on page 2-19

Overview

High-throughput sequencing instruments produce large amounts of sequence read data that can be challenging 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 sequence read data.

<table>
<thead>
<tr>
<th>Object</th>
<th>Contains This Information</th>
<th>Construct from One of These</th>
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</thead>
<tbody>
<tr>
<td>BioRead</td>
<td>• Sequence headers&lt;br&gt; • Read sequences&lt;br&gt; • Sequence qualities (base calling)</td>
<td>• FASTQ file&lt;br&gt; • SAM file&lt;br&gt; • FASTQ structure (created using the <code>fastqread</code> function)&lt;br&gt; • SAM structure (created using the <code>samread</code> function)&lt;br&gt; • Cell arrays containing header, sequence, and quality information (created using the <code>fastqread</code> function)</td>
</tr>
<tr>
<td>Object</td>
<td>Contains This Information</td>
<td>Construct from One of These</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| BioMap   | • Sequence headers  
• Read sequences  
• Sequence qualities (base calling)  
• Sequence alignment and mapping information (relative to a single reference sequence), including mapping quality                                                                                      | • SAM file  
• BAM file  
• SAM structure (created using the `samread` function)  
• BAM structure (created using the `bamread` function)  
• Cell arrays containing header, sequence, quality, and mapping/alignment information (created using the `samread` or `bamread` function) |

**Represent Sequence and Quality Data in a BioRead Object**

**Prerequisites**

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

Construct a BioRead object in one of two ways:

- **Indexed** — The data remains in the source file. Constructing the object and accessing its contents is memory efficient. However, you cannot modify object properties, other than the Name property. This is the default method if you construct a BioRead object from a FASTQ- or SAM-formatted file.

- **In Memory** — The data is read into memory. Constructing the object and accessing its contents is limited by the amount of available memory. However, you can modify object properties. When you construct a BioRead object from a FASTQ structure or cell arrays, the data is read into memory. When you construct a BioRead object from a FASTQ- or SAM-formatted file, use the InMemory name-value pair argument to read the data into memory.

**Construct a BioRead Object from a FASTQ- or SAM-Formatted File**

**Note** This example constructs a BioRead object from a FASTQ-formatted file. Use similar steps to construct a BioRead object from a SAM-formatted file.
Use the **BioRead** constructor function to construct a **BioRead** object from a FASTQ-formatted file and set the **Name** property:

\[\text{BRObj1} = \text{BioRead('SRR005164_1_50.fastq', 'Name', 'MyObject')}\]

**BRObj1** =

**BioRead** with properties:

- **Quality**: [50x1 File indexed property]
- **Sequence**: [50x1 File indexed property]
- **Header**: [50x1 File indexed property]
- **NSeqs**: 50
- **Name**: 'MyObject'

The constructor function construct a **BioRead** object and, if an index file does not already exist, it also creates an index file with the same file name, but with an .IDX extension. This index file, by default, is stored in the same location as the source file.

**Caution** Your source file and index file must always be in sync.

- After constructing a **BioRead** object, do not modify the index file, or you can get invalid results when using the existing object or constructing new objects.
- If you modify the source file, delete the index file, so the object constructor creates a new index file when constructing new objects.

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

### Represent Sequence, Quality, and Alignment/Mapping Data in a **BioMap** Object

**Prerequisites**

A **BioMap** object represents a collection of sequence reads 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.
When constructing a BioMap object from a BAM file, the maximum size of the file is limited by your operating system and available memory.

Construct a BioMap object in one of two ways:

- **Indexed** — The data remains in the source file. Constructing the object and accessing its contents is memory efficient. However, you cannot modify object properties, other than the Name property. This is the default method if you construct a BioMap object from a SAM- or BAM-formatted file.

- **In Memory** — The data is read into memory. Constructing the object and accessing its contents is limited by the amount of available memory. However, you can modify object properties. When you construct a BioMap object from a structure, the data stays in memory. When you construct a BioMap object from a SAM- or BAM-formatted file, use the InMemory name-value pair argument to read the data into memory.

### Construct a BioMap Object from a SAM- or BAM-Formatted File

**Note** This example constructs a BioMap object from a SAM-formatted file. Use similar steps to construct a BioMap object from a BAM-formatted file.

1. If you do not know the number and names of the reference sequences in your source file, determine them using the saminfo or baminfo function and the ScanDictionary name-value pair argument.

   ```matlab
   samstruct = saminfo('ex2.sam', 'ScanDictionary', true);
   samstruct.ScannedDictionary
   ans =
         'seq1'
         'seq2'
   ```

   **Tip** The previous syntax scans the entire SAM file, which is time consuming. If you are confident that the Header information of the SAM file is correct, omit the ScanDictionary name-value pair argument, and inspect the SequenceDictionary field instead.

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

BMObj2 = BioMap('ex2.sam', 'SelectRef', 'seq1', 'Name', 'MyObject')

BMObj2 =

BioMap with properties:

SequenceDictionary: 'seq1'
  Reference: [1501x1 File indexed property]
  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: 'MyObject'

The constructor function constructs a BioMap object and, if index files do not already exist, it also creates one or two index files:

- If constructing from a SAM-formatted file, it creates one index file that has the same file name as the source file, but with an .IDX extension. This index file, by default, is stored in the same location as the source file.
- If constructing from a BAM-formatted file, it creates two index files that have the same file name as the source file, but one with a .BAI extension and one with a .LINEARINDEX extension. These index files, by default, are stored in the same location as the source file.

**Caution** Your source file and index files must always be in sync.

- After constructing a BioMap object, do not modify the index files, or you can get invalid results when using the existing object or constructing new objects.
- If you modify the source file, delete the index files, so the object constructor creates new index files when constructing new objects.
**Note** Because you constructed this BioMap object from a source file, you cannot modify the properties (except for Name and Reference) of the BioMap object.

**Construct a BioMap Object from a SAM or BAM Structure**

**Note** This example constructs a BioMap object from a SAM structure using `samread`. Use similar steps to construct a BioMap object from a BAM structure using `bamread`.

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

   ```matlab
   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:

   ```matlab
disp(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 `SelectRef` name-value pair argument to specify one reference sequence, `seq1`:

   ```matlab
   BMObj1 = BioMap(SAMStruct, 'SelectRef', 'seq1')
   ```

   ```matlab
   BMObj1 = BioMap with properties:
   SequenceDictionary: {'seq1'}
   Reference: {1501x1 cell}
   Signature: {1501x1 cell}
   Start: [1501x1 uint32]
   MappingQuality: [1501x1 uint8]
   Flag: [1501x1 uint16]
   MatePosition: [1501x1 uint32]
   Quality: {1501x1 cell}
   Sequence: {1501x1 cell}
   Header: {1501x1 cell}
   ```
Retrieve Information from a BioRead or BioMap Object

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

Retrieve a Property from a BioRead or BioMap Object

You can retrieve a specific property from elements in a BioRead or BioMap object.

For example, to retrieve all headers from a BioRead object, use the Header property as follows:

```matlab
allHeaders = BRObj1.Header;
```

This syntax returns a cell array containing the headers for all elements in the BioRead object.

Similarly, to retrieve all start positions of aligned read sequences from a BioMap object, use the Start property of the object:

```matlab
allStarts = BMObj1.Start;
```

This 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.

Retrieve Multiple Properties from a BioRead or BioMap Object

You can retrieve multiple properties from a BioRead or BioMap object in a single command using the get method. For example, to retrieve both start positions and headers information of a BioMap object, use the get method as follows:

```matlab
multiProp = get(BMObj1, {'Start', 'Header'});
```

This syntax returns a cell array containing all start positions and headers information of 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.
Retrieve 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:

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

This 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:

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

```
subSeqs =
    'TGGCTTTAAAGC'
    'CCCGAAAGCTAG'
    'AATTTTGC GGCT'
```

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

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

This syntax returns a tab-delimited character vector 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 character vector) 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.

Set Information in a BioRead or BioMap Object

Prerequisites

To modify properties (other than Name and Reference) of a BioRead or BioMap object, the data must be in memory, and not indexed. To ensure the data is in memory, do one of the following:

- Construct the object from a structure as described in “Construct a BioMap Object from a SAM or BAM Structure” on page 2-13.
- Construct the object from a source file using the InMemory name-value pair argument.

Provide Custom Headers for Sequences

First, create an object with the data in memory:

```matlab
BRObj1 = BioRead('SRR005164_1_50.fastq','InMemory',true);
```

To provide custom headers for sequences of interest (in this case sequences 1 to 5), do the following:

```matlab
BRObj1.Header(1:5) = {'H1', 'H2', 'H3', 'H4', 'H5'};
```

Alternatively, you can use the setHeader method:

```matlab
BRObj1 = setHeader(BRObj1, {'H1', 'H2', 'H3', 'H4', 'H5'}, [1:5]);
```

Several other specialized set methods let you set the properties of a subset of elements in a BioRead or 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.
**Determine 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:

```plaintext
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
```
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:

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

```plaintext
Cov =
     1     1     2     2     3     4     4     4     5     5
```

**Construct 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:

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

```plaintext
Alignment_1_12 =
CACTAGTG
CTAGTG
AGTGG
GTGG
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'

Filter Read Sequences Using SAM Flags

SAM- and BAM-formatted files include the status of 11 binary 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.

Filter Unmapped Read Sequences


   BMObj2 = BioMap('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);
Filter Read Sequences That Are Not Mapped in a Pair


   ```matlab
   BMObj2 = BioMap('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.

   ```matlab
   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.

   ```matlab
   filteredBMObj_2 = getSubset(BMObj2, LogicalVec_paired);
   ```
Represent Feature Annotations in a GFFAnnotation or GTFAnnotation Object

The GFFAnnotation and GTFAnnotation objects represent a collection of feature annotations for one or more reference sequences. You construct these objects from GFF (General Feature Format) and GTF (Gene Transfer Format) files. Each element in the object represents a single annotation. The properties and methods associated with the objects let you investigate and filter the data based on reference sequence, a feature (such as CDS or exon), or a specific gene or transcript.

Construct an Annotation Object

Use the GFFAnnotation constructor function to construct a GFFAnnotation object from either a GFF- or GTF-formatted file:

GFFAnnotObj = GFFAnnotation('tair8_1.gff')

GFFAnnotObj =

GFFAnnotation with properties:

FieldNames: {1x9 cell}
NumEntries: 3331

Use the GTFAnnotation constructor function to construct a GTFAnnotation object from a GTF-formatted file:

GTFAnnotObj = GTFAnnotation('hum37_2_1M.gtf')
GTFAnnotObj =

GTFAnnotation with properties:

FieldNames: {1x11 cell}
NumEntries: 308

**Retrieve General Information from an Annotation Object**

Determine the field names and the number of entries in an annotation object by accessing the FieldNames and NumEntries properties. For example, to see the field names for each annotation object constructed in the previous section, query the FieldNames property:

GFFAnnotObj.FieldNames
ans =

Columns 1 through 6
'Reference'  'Start'  'Stop'  'Feature'  'Source'  'Score'
Columns 7 through 9
'Strand'  'Frame'  'Attributes'
GTFAnnotObj.FieldNames
ans =

Columns 1 through 6
'Reference'  'Start'  'Stop'  'Feature'  'Gene'  'Transcript'
Columns 7 through 11
'Source'  'Score'  'Strand'  'Frame'  'Attributes'

Determine the range of the reference sequences that are covered by feature annotations by using the getRange method with the annotation object constructed in the previous section:

range = getRange(GFFAnnotObj)
range =

    3631    498516

**Access Data in an Annotation Object**

**Create a Structure of the Annotation Data**

Creating a structure of the annotation data lets you access the field values. Use the `getData` method to create a structure containing a subset of the data in a `GFFAnnotation` object constructed in the previous section.

% Extract annotations for positions 1 through 10000 of the reference sequence
AnnotStruct = getData(GFFAnnotObj,1,10000)

AnnotStruct =

60x1 struct array with fields:
  Reference
  Start
  Stop
  Feature
  Source
  Score
  Strand
  Frame
  Attributes

**Access Field Values in the Structure**

Use dot indexing to access all or specific field values in a structure.

For example, extract the start positions for all annotations:

Starts = AnnotStruct.Start;

Extract the start positions for annotations 12 through 17. Notice that you must use square brackets when indexing a range of positions:

Starts_12_17 = [AnnotStruct(12:17).Start]

Starts_12_17 =

    4706  5174  5174  5439  5439  5631
Extract the start position and the feature for the 12th annotation:

\[
\text{Start}_{12} = \text{AnnotStruct}(12).\text{Start}
\]

\[
\text{Start}_{12} = 4706
\]

\[
\text{Feature}_{12} = \text{AnnotStruct}(12).\text{Feature}
\]

\[
\text{Feature}_{12} = \text{CDS}
\]

Use Feature Annotations with Sequence Read Data

Investigate the results of HTS sequencing experiments by using \text{GFFAnnotation} and \text{GTFAnnotation} objects with \text{BioMap} objects. For example, you can:

- Determine counts of sequence reads aligned to regions of a reference sequence associated with specific annotations, such as in RNA-Seq workflows.
- Find annotations within a specific range of a peak of interest in a reference sequence, such as in ChIP-Seq workflows.

Determine Annotations of Interest

1. Construct a \text{GTFAnnotation} object from a GTF-formatted file:

\[
\text{GTFAnnotObj} = \text{GTFAnnotation}('\text{hum37}_2_1M.gtf');
\]

2. Use the \text{getReferenceNames} method to return the names for the reference sequences for the annotation object:

\[
\text{refNames} = \text{getReferenceNames}(	ext{GTFAnnotObj})
\]

\[
\text{refNames} = \text{chr2}
\]

3. Use the \text{getFeatureNames} method to retrieve the feature names from the annotation object:

\[
\text{featureNames} = \text{getFeatureNames}(	ext{GTFAnnotObj})
\]

\[
\text{featureNames} = \text{CDS}
\]
Use the `getGeneNames` method to retrieve a list of the unique gene names from the annotation object:

```python
geneNames = getGeneNames(GTFAnnotObj)
geneNames =

'uc002qvu.2'
'uc002qvv.2'
'uc002qvw.2'
'uc002qvx.2'
'uc002qvy.2'
'uc002qvz.2'
'uc002qwa.2'
'uc002qwb.2'
'uc002qwc.1'
'uc002qwd.2'
'uc002qwe.3'
'uc002qwf.2'
'uc002qwg.2'
'uc002qwh.2'
'uc002qwi.3'
'uc002qwk.2'
'uc002qwl.2'
'uc002qwm.1'
'uc002qwn.1'
'uc002qwo.1'
'uc002qwp.2'
'uc002qwq.2'
'uc010ewe.2'
'uc010ewf.1'
'uc010ewg.2'
'uc010ewh.1'
'uc010ewi.2'
'uc010yim.1'
```

The previous steps gave us a list of available reference sequences, features, and genes associated with the available annotations. Use this information to determine annotations of interest. For instance, you might be interested only in annotations that are exons associated with the `uc002qvv.2` gene on chromosome 2.
**Filter Annotations**

Use the `getData` method to filter the annotations and create a structure containing only the annotations of interest, which are annotations that are exons associated with the `uc002qvv.2` gene on chromosome 2.

\[
\text{AnnotStruct} = \text{getData(GTFAnnotObj,'Reference','chr2',...}
\quad \text{'Feature','exon','Gene','uc002qvv.2')}
\]

\[
\text{AnnotStruct} =
\]

12x1 struct array with fields:
- Reference
- Start
- Stop
- Feature
- Gene
- Transcript
- Source
- Score
- Strand
- Frame
- Attributes

The return structure contains 12 elements, indicating there are 12 annotations that meet your filter criteria.

**Extract Position Ranges for Annotations of Interest**

After filtering the data to include only annotations that are exons associated with the `uc002qvv.2` gene on chromosome 2, use the Start and Stop fields to create vectors of the start and end positions for the ranges associated with the 12 annotations.

\[
\text{StartPos} = \text{[AnnotStruct.Start]};
\]

\[
\text{EndPos} = \text{[AnnotStruct.Stop]};
\]

**Determine Counts of Sequence Reads Aligned to Annotations**

Construct a `BioMap` object from a BAM-formatted file containing sequence read data aligned to chromosome 2.

\[
\text{BMObj3} = \text{BioMap('ex3.bam')};
\]
Then use the range for the annotations of interest as input to the `getCounts` method of a `BioMap` object. This returns the counts of short reads aligned to the annotations of interest.

```plaintext
counts = getCounts(BMObj3,StartPos,EndPos,'independent', true)
counts =
  1399
   1
   54
  221
   97
  125
   0
   1
   0
  65
   9
  12
```
Visualize and Investigate Sequence Read Alignments

When to Use the NGS Browser to Visualize and Investigate Data

The NGS Browser lets you visually verify and investigate the alignment of sequence reads to a reference sequence, in support of analyses that measure genetic variations and gene expression. The NGS Browser lets you:

- Visualize sequence reads aligned to a nucleotide reference sequence.
- Compare multiple data sets aligned against a common reference sequence.
- View coverage of different bases and regions of the reference sequence.
- Investigate quality and other details of aligned reads.
- Identify mismatches due to base-calling errors or polymorphisms.
- Visualize insertions and deletions.
- Retrieve feature annotations relative to a specific region of the reference sequence.
- Investigate regions of interest in the alignment, determined by various analyses.
You can visualize and investigate the aligned data before, during, or after any preprocessing (filtering, quality recalibration) or analysis steps you perform on the aligned data.

**Open the NGS Browser**

To open the **NGS Browser**, type the following in the MATLAB Command Window:

```matlab
ngsbrowser
```

Alternatively, click the **NGS Browser** on the **Apps** tab.
Import Data into the NGS Browser

Ruler indicates maximum coverage in display range

Rubberband indicates range displayed in 3 tracks

Browser Displaying Reference Track, One Alignment Track, and One Annotation Track

Import a Reference Sequence

You can import a single reference sequence into the NGS Browser. The reference sequence must be in a FASTA file.
1. Select **File > Add Data from File**.
2. In the Open dialog box, select a FASTA file, and then click **Open**.

**Tip** You can use the `getgenbank` function with the `ToFile` and `SequenceOnly` name-value pair arguments to retrieve a reference sequence from the GenBank database and save it to a FASTA-formatted file.

### Import Sequence Read Alignment Data

You can import multiple data sets of sequence read alignment data. The alignment data must be in either of the following:

- **BioMap object**

  **Tip** Construct a BioMap object on page 2-10 from a SAM- or BAM-formatted file to investigate, subset, and filter on page 2-19 the data before importing it into the NGS Browser.

- **SAM- or BAM-formatted file**

  **Note** Your SAM- or BAM-formatted file must:

  - Have reads ordered by start position in the reference sequence.
  - Have an IDX index file (for a SAM-formatted file) or BAI and LINEARINDEX index files (for a BAM-formatted file) stored in the same location as your source file. Otherwise, the source file must be stored in a location to which you have write access, because MATLAB needs to create and store index files in this location.

  **Tip** Try using SAMtools to check if the reads in your SAM- or BAM-formatted file are ordered by position in the reference sequence, and also to reorder them, if needed.

  **Tip** If you do not have index files (IDX or BAI and LINEARINDEX) stored in the same location as your source file, and your source file is stored in a location to which you do not have write access, you cannot import data from the source file directly into the browser. Instead, construct a BioMap object from the source file using the `IndexDir` name-value pair argument, and then import the BioMap object into the browser.
To import sequence read alignment data:

1. Select **File > Add Data from File** or **File > Import Alignment Data from MATLAB Workspace**.
2. Select a SAM-formatted file, BAM-formatted file, or BioMap object.
3. If you select a file containing multiple reference sequences, in the Select Reference dialog box, select a reference or scan the file for available references and their mapped reads counts. Click **OK**.
4. Repeat the previous steps to import additional data sets.

**Import Feature Annotations**

You can import multiple sets of feature annotations from GFF- or GTF-formatted files that contain data for a single reference sequence.

1. Select **File > Add Data from File**.
2. In the Open dialog box, select a GFF- or GTF-formatted file, and then click **Open**.
3. Repeat the previous steps to import additional annotations.

**Zoom and Pan to a Specific Region of the Alignment**

To zoom in and out:

Use the toolbar buttons, or click-drag an edge of the rubberband in the Overview area.

To pan across the alignment:

Use the toolbar buttons, or click-drag the rubberband in the Overview area.

**Tip** Use the left and right arrow keys to pan in one base pair (bp) increments.
View Coverage of the Reference Sequence

At the top of each alignment track, the *coverage view* displays the coverage of each base in the reference sequence. The vertical ruler on the left edge of the coverage view indicates the maximum coverage in the display range. Hover the mouse pointer over a position in the coverage view to display the location and counts.

**Note** The browser computes coverage at the base pair resolution, instead of binning, even when zoomed out.

To change the percent coverage displayed, click anywhere in the alignment track, and then edit the Alignment Coverage settings.

**Tip** Set **Max** to a value greater than 100, if needed, when comparing the coverage of multiple tracks of reads.

View the Pileup View of Short Reads

Each alignment track includes a *pileup view* of the short reads aligned to the reference sequence.
Limit the depth of the reads displayed in the pileup view by setting the **Maximum display read depth** in the Alignment Pileup settings.

**Tip** Limiting the depth of short reads in the pileup view does not change the counts displayed in the coverage view.

**Compare Alignments of Multiple Data Sets**

Compare multiple data sets, with each data set in its own track, against a common reference sequence. Use the **Track List** to show/hide, order, and delete tracks of data.
View Location, Quality Scores, and Mapping Information

Hover the mouse pointer over a position in a read to display strand direction, location, quality, and mapping information for the base, the read, and its paired mate.
Flag Reads

Click anywhere in an alignment track to display the Alignment Pileup settings.
Flag Reads with Low Mapping Quality

Set the **Mapping quality threshold** in the Alignment Pileup section to flag low-quality reads. Reads with a mapping quality below this level appear in a lighter shade of gray.

Flag Duplicate Reads

Select **Flag duplicate reads** and select an outline color.

Flag Reads with Unmapped Pairs

Select **Flag reads with unmapped pair** and select an outline color.

Evaluate and Flag Mismatches

Mismatches display as colored blocks or letters, depending on the zoom level.

![Zoomed out view of read — Mismatches display as bars](image)

![Zoomed in view of read — Mismatches display as letters](image)

In addition to the base Phred quality information that displays in the tooltip, you can visualize quality differences by using the **Shade mismatch bases by Phred quality** settings.

![Shade mismatch bases by Phred quality:](image)

The mismatch blocks or letters display in:

- Light shade — Mismatch bases with Phred scores below the minimum
• Graduation of medium shades — Mismatch bases with Phred scores within the minimum to maximum range
• Dark shade — Mismatch bases with Phred scores above the maximum

**View Insertions and Deletions**

The NGS Browser designates insertions with a symbol. Hover the mouse pointer over the insertion symbol to display information about it.

![Insertion Example]

The NGS Browser designates deletions with dashes.

![Deletion Example]

**View Feature Annotations**

After importing a feature annotation file, you can zoom and pan to view feature annotations associated with a region of interest in the alignment. Hover the mouse pointer over the feature annotation.

![Feature Annotation Example]
Print and Export the Browser Image

Print or export the browser image by selecting **File > Print Image** or **File > Export Image**.
Count Features from NGS Reads

This example shows how to count features from paired-end sequencing reads after aligning them to the whole human genome curated by the Genome Reference Consortium. This example uses Genome Reference Consortium Human Build 38 patch release 12 (GRCh38.p12) as the human genome reference.

Prerequisites and Data Set

This example works on the UNIX® and Mac platforms only. Download the Bioinformatics Toolbox™ Interface for Bowtie Aligner support package from the Add-On Explorer.

This example assumes you have:

- Downloaded and extracted the RefSeq assembly from Genome Reference Consortium Human Build 38 patch release 12 (GRCh38.p12).
- Downloaded and organized some paired-end reads data. This example uses the exome sequencing data from the 1000 genomes project. Paired-end reads are indicated by '1' and '2' in the filenames following the accession number. Here is one possibility for how the data can be organized in folders:

```
sequence/
  |--HG00096/
  |    |--SRR077487_1.filt.fastq
  |    |--SRR077487_2.filt.fastq
  |    |--SRR081241_1.filt.fastq
  |    |--SRR081241_2.filt.fastq
  |
  |--HG00097
  |    |-- SRR765989_1.filt.fastq
  |    |-- SRR765989_2.filt.fastq
```

Build Index

Construct an index for aligning reads to the reference using `bowtie2build`. The file `GCF_000001405.38_GRCh38.p12_genomic.fna` contains the human reference genome in the FASTA format. `bowtieIdx` is the base name of the reference index files. The `--threads 8` option specifies the number of parallel threads to build index files faster.

```
bowtieIdx  = 'GCF_000001405.38_GRCh38.p12_genomic.index';
bUILDFlag  = bowtie2build('GCF_000001405.38_GRCh38.p12_genomic.fna',...
                          bowtieIdx,'--threads 8');
```
Align Reads to Reference

Use the helper function `alignAllReads` to align each set of paired-end reads to the reference. The function produces a SAM file in the current folder for each sample in the 'sequence' folder.

```
addpath(fullfile(matlabroot,'examples','bioinfo','main')); % Make sure the supporting files are on the search path
type alignAllReads
```

```
function samFiles = alignAllReads(indexBaseName, inputDir, outputDir)
    %ALIGNALLREADS Helper function for CountFeaturesExample
    %
    % SAMFILES = alignAllReads(INDEXBASENAME, INPUTDIR, OUTPUTDIR) aligns
    % paired-end sequencing reads using Bowtie2 to a prebuilt index,
    % producing SAM-formatted alignment files. INDEXBASENAME
    % is a character vector specifying the prefix of the index files
    % created with BOWTIE2BUILD. INPUTDIR contains subdirectories for each
    % sample containing paired-end reads in FASTQ format:
    %
    % INPUTDIR/
    % +-- HG00096/
    %   |   +-- SRR077487_1.filt.fastq
    %   |   +-- SRR077487_2.filt.fastq
    %   |   +-- SRR081241_1.filt.fastq
    %   |   +-- SRR081241_2.filt.fastq
    %   |
    %   +-- HG00097/
    %     +-- SRR765989_1.filt.fastq
    %     +-- SRR765989_2.filt.fastq
    %
    % NOTE: each mate is distinguished by the '_1' or '_2' after the
    % accession number in the filename.
    %
    % SAMFILES is a cell array of the resulting SAM-formatted files created
    % with Bowtie2, and are placed in OUTPUTDIR.
    %
    % Copyright 2018 The MathWorks, Inc.
    %
    % Use dir() to identify sample names (subfolders of inputDir).
    d = dir(inputDir);
    samples = {d(3:end).name}; % skip special '.' & '..' folders
    samFiles = strcat(samples, '.sam');

    for s=1:numel(samples)
```

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% Identify mate pairs of reads for each sample
sampleReadsPath = fullfile(inputDir, samples{s});
reads1 = dir(fullfile(sampleReadsPath, '/*_1*'));
reads2 = dir(fullfile(sampleReadsPath, '/*_2*'));

reads1 = fullfile(sampleReadsPath, {reads1(:).name});
reads2 = fullfile(sampleReadsPath, {reads2(:).name});

% Get full filename to SAM file
samFiles{s} = fullfile(outputDir, samFiles{s});

% Perform alignment, if file doesn't exist
if ~exist(samFiles{s}, 'file')
    bowtie2(indexBaseName, reads1, reads2, samFiles{s}, '-p 2');
end
end
end

samFiles = alignAllReads(bowtieIdx,'sequence','.');

Selectively Align to Gene of Interest

SAM files can be very large. Use BioMap to select only the data for the correct reference. For this example, consider APOE, which is a gene on Chromosome 19 linked to Alzheimer's disease. Create a set of smaller BAM files for APOE to improve performance.

apoeRef = 'NC_000019.10'; % Reference name for Chromosome 19 in HG38
bamFiles = strrep(samFiles,'.sam','.bam');
for i=1:numel(samFiles)
    if ~exist(bamFiles{i},'file')
        bm = BioMap(samFiles{i},'SelectReference',apoeRef);
        write(bm, bamFiles{i},'Format','bam');
    end
end

Summarize Read Counts

Use featurecount to compare the number of transcripts for each APOE variant using a GTF file. A full table of features is included in the GRCh38.p12 assembly in GFF format, which can be converted to GTF using cuffgffread. This example uses a simplified GTF based on APOE transcripts. AP0E_gene.gtf is included with the software.

[FeatTable, Summary] = featurecount('APOE_gene.gtf',bamFiles,...
    'Metafeature','transcript_id');
Processing GTF file APOE_gene.gtf ...
Processing BAM file ./HG00096.bam ...
Processing reference NC_000019.10 ...
10000 reads processed ...
20000 reads processed ...
30000 reads processed ...
40000 reads processed ...
50000 reads processed ...
60000 reads processed ...
70000 reads processed ...
80000 reads processed ...
90000 reads processed ...
100000 reads processed ...
110000 reads processed ...
120000 reads processed ...
130000 reads processed ...
140000 reads processed ...
150000 reads processed ...
160000 reads processed ...
170000 reads processed ...
180000 reads processed ...
190000 reads processed ...
200000 reads processed ...
210000 reads processed ...
220000 reads processed ...
230000 reads processed ...
240000 reads processed ...
250000 reads processed ...
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1300000 reads processed ...
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2530000 reads processed ...
2540000 reads processed ...
2550000 reads processed ...
2560000 reads processed ...
2570000 reads processed ...
2580000 reads processed ...
2590000 reads processed ...
2600000 reads processed ...
2610000 reads processed ...
Count Features from NGS Reads

2620000 reads processed ...
2630000 reads processed ...
2640000 reads processed ...
2650000 reads processed ...
2660000 reads processed ...
2670000 reads processed ...
2680000 reads processed ...
2690000 reads processed ...
2700000 reads processed ...
2710000 reads processed ...
2720000 reads processed ...
2730000 reads processed ...
2740000 reads processed ...
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2760000 reads processed ...
2770000 reads processed ...
2780000 reads processed ...
2790000 reads processed ...
2800000 reads processed ...
2810000 reads processed ...
2820000 reads processed ...
2830000 reads processed ...
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2860000 reads processed ...
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2900000 reads processed ...
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2930000 reads processed ...
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2990000 reads processed ...
3000000 reads processed ...
3010000 reads processed ...
3020000 reads processed ...
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3040000 reads processed ...
3050000 reads processed ...

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3500000 reads processed ...
3510000 reads processed ...
3520000 reads processed ...
3530000 reads processed ...
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3560000 reads processed ...
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3580000 reads processed ...
3590000 reads processed ...
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3810000 reads processed ...
3820000 reads processed ...
3830000 reads processed ...
3840000 reads processed ...
3850000 reads processed ...
3860000 reads processed ...
3870000 reads processed ...
3880000 reads processed ...
3890000 reads processed ...
3900000 reads processed ...
3910000 reads processed ...
3920000 reads processed ...
3930000 reads processed ...

Count Features from NGS Reads
3940000 reads processed ... 
3950000 reads processed ... 
3960000 reads processed ... 
3970000 reads processed ... 
Done.

See Also
BioMap | bowtie2 | bowtie2build | cuffgffread | cufflinks | featurecount
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.

- “Exploring a Nucleotide Sequence Using Command Line” on page 3-2
- “Exploring a Nucleotide Sequence Using the Sequence Viewer App” on page 3-20
- “Explore a Protein Sequence Using the Sequence Viewer App” on page 3-35
- “Compare Sequences Using Sequence Alignment Algorithms” on page 3-40
- “View and Align Multiple Sequences” on page 3-58
Exploring a Nucleotide Sequence Using Command Line

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<tr>
<td>“Reading Sequence Information from the Web” on page 3-5</td>
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<tr>
<td>“Determining Nucleotide Composition” on page 3-6</td>
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<td>“Determining Codon Composition” on page 3-10</td>
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<tr>
<td>“Open Reading Frames” on page 3-15</td>
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<tr>
<td>“Amino Acid Conversion and Composition” on page 3-17</td>
</tr>
</tbody>
</table>

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.

Searching the Web for Sequence Information

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

```matlab
```

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.

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.
### Genome > Eukaryota > Homo sapiens mitochondrion, complete genome

**Lineage:** Eukaryota, Fungi/Metazoa group, Metazoa, Eumetazoa, Bilateria, Coeloctea, Deuterostomia, Chordata, Craniata, Vertebrata, Gnathostomata, Teleostomi, Euteleostomi, Sarcopterygi, Tetrapoda, Amniota, Mammalia, Theria, Eutheria, Euarchontoglires, Primates, Haplorhini, Simiformes, Catarrhini, Hominidae, Hominoidea, Hominins, Homo, Homo sapiens

<table>
<thead>
<tr>
<th>Genome Info</th>
<th>Features</th>
<th>BLAST homologs</th>
<th>Links</th>
<th>Review Info</th>
</tr>
</thead>
<tbody>
<tr>
<td>RefSeq NC_012920</td>
<td>Genes: 37</td>
<td>COG</td>
<td>Genome Project</td>
<td>Publications: 2</td>
</tr>
<tr>
<td>GenBank JQ1418</td>
<td>Protein coding: 13</td>
<td>TaxMap</td>
<td>RefSeq FTP</td>
<td>RefSeq Status: PROVISIONAL</td>
</tr>
<tr>
<td>Length: 16,569 nt</td>
<td>Structural RNAs: 24</td>
<td>TaxPlot</td>
<td>GenBank FTP</td>
<td>Seq. Status: Completed</td>
</tr>
<tr>
<td>GC Content: 44%</td>
<td>Pseudo genes: None</td>
<td>GenePlot</td>
<td>BLAST</td>
<td>Sequencing center: Center for Molecular and Mitochondrial Medicine and Genetics (CMMAC), University of California, Irvine, Mitomap.org, USA, Irvine</td>
</tr>
<tr>
<td>% Coding: 58%</td>
<td>Others: 30</td>
<td>gMap</td>
<td>TraceAssembly</td>
<td>Completed: 2009/07/08</td>
</tr>
<tr>
<td>Topology: circular</td>
<td>Configs: None</td>
<td>COD</td>
<td>Organism Group</td>
<td></td>
</tr>
<tr>
<td>Molecule: dsDNA</td>
<td>Other genomes for species: 8883</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Gene Classification based on COG functional categories

Search gene, GeneID or locus_tag: Find Gene

[Click here for Sequence Viewer presentation (base sequence and aligned amino acids) of selected region]
Reading Sequence Information from the Web

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 = 
GATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCTCTCCATGCAT
TTGGTATTTTCGTCTGGGGGGTGTGCACGCGATAGCATTGCGAGACGCTG
GAGCCGGAGCACCCTATGTCGCAGTATCTGTCTTTGATTCCTGCCTCATT
CTATTATTTATCGCACCCTACGTTCAATATTACAGGCGAACATACCTACTA
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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Size</th>
<th>Bytes</th>
<th>Class</th>
<th>Attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td>mitochondria</td>
<td>1x16569</td>
<td>33138</td>
<td>char</td>
<td></td>
</tr>
</tbody>
</table>
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 from the Web” on page 3-5.

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

\[ \text{ndensity(mitochondria)} \]

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 from the Web” on page 3-5.

1  Count codons in a nucleotide sequence. In the MATLAB Command Window, type
codoncount(mitochondria)

The codon counts for the first reading frame displays.
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 from the Web” on page 3-5.

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

```matlab
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.

2 Display ORFs using the Vertebrate Mitochondrial code.

```matlab
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.

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

The stop position displays.
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.

```
attaatccctggcccaacccgctcatctactctaccatctttgcaggcac
actcatcacagcgtgctagctgcactgattttttacctgagtaggcctag
aaataaacatgctagcttttatttttatccagtttctaaccckaaaaaaaataaacccct
cgtccacagaagctgcctcaagtatttctcagcaagcaacccgcctc
cataatccttc . . .
```

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 - 1
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
```

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 3-15.

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.

   MNPLAQPVYSTIFAGTLITALSSHWFTWVGLEMNLAFIPVLTKKMN
   RSTEAIAKYFLQTATASMILMAILFNNMLSGQWTMTNTTNQYSSLMIMM
   AMAMKLGMAPFHVPEVTQGTLTSLLLLTWQKLAPSIMYQISPRLN
   VSLLLTSSLSCMGSWGRNQTLKILAYSSITHMGWMMAVLPYPNM
   TILNLITYIILTTTAFLLNLNLSSTTTCRLTWNKLTWLTPLIPSTLLLS
   LGGLPPPLTGFLPKWAIIEFTKNSLIIPTITMATITLNLNYFYLRLIYST
   SITLLPMSNVMKMKWQFEHTKPTPFLPTLIALTLLLPISPFMLMIL

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
```
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.
Exploring a Nucleotide Sequence Using the Sequence Viewer App

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<td>“Importing a Sequence into the Sequence Viewer” on page 3-20</td>
</tr>
<tr>
<td>“Viewing Nucleotide Sequence Information” on page 3-23</td>
</tr>
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<td>“Searching for Words” on page 3-27</td>
</tr>
<tr>
<td>“Exploring Open Reading Frames” on page 3-29</td>
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<tr>
<td>“Closing the Sequence Viewer” on page 3-34</td>
</tr>
</tbody>
</table>

Overview of the Sequence Viewer

The **Sequence Viewer** integrates many of the sequence functions in the Bioinformatics Toolbox toolbox. Instead of entering commands in the MATLAB Command Window, you can select and enter options using the app.

Importing a Sequence into the Sequence Viewer

The first step when analyzing a nucleotide or amino acid sequence is to import sequence information into the MATLAB environment. The **Sequence Viewer** 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.

**Note**  Data in public repositories is frequently curated and updated; therefore, the results of this example might be slightly different when you use up-to-date sequences.

1. In the MATLAB Command Window, type
   ```matlab
   seqviewer
   ```
   Alternatively, click **Sequence Viewer** on the **Apps** tab.
The **Sequence Viewer** 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 Viewer app, 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.
Table: Sequence Analysis

<table>
<thead>
<tr>
<th>Position</th>
<th>Word</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>tcacataca acaagtttg gtttaatcc ctcgtttttc tgtctgtgaa gtaaccttag</td>
</tr>
<tr>
<td>61</td>
<td></td>
<td>ccggccaagt cccctaacct cccgtaggccc tggcgaggtg cacacaaca a tcacagttt</td>
</tr>
<tr>
<td>121</td>
<td></td>
<td>acactaggg cacttggaa aactttttct cagaggtggtg ctagtgttctt gactttcace</td>
</tr>
<tr>
<td>181</td>
<td></td>
<td>ccggggttct ctagcggggt tgaagttgct aagttcataa ccggcccggga aacgtcaggg</td>
</tr>
<tr>
<td>241</td>
<td></td>
<td>gtggcggttt atttaactgtc taactggggc ctggacaggt cggcgaggt cagacagtttg</td>
</tr>
<tr>
<td>301</td>
<td></td>
<td>ccaacccccc cacaatggcc tggcccaggg aagcatcctt gttcaaggg ggattgttaaa</td>
</tr>
</tbody>
</table>

Base Count:
- A: 593 (21.6%)
- C: 750 (27.3%)
- G: 716 (26.0%)
- T: 692 (23.2%)

Map View:
- NM_000520: Homo sapiens hexosaminidase subunit alpha (HEXA) transcript variant 2, mRNA.
- Words found: 33
- 2751 bp

4.7 BP/Pixel
4 Click **Annotated CDS** to show the protein coding part of a nucleotide sequence.
Searching for Words

You can also search for characteristic words or sequence patterns using regular expressions. You can enter the IUB/IUPAC nucleotide and amino acid symbols that are automatically converted to corresponding nucleotides and amino acids accordingly. For details about how symbols are interpreted, see the Nucleotide Conversion and Amino Acid Conversion tables of seq2regexp. For instance, if you search for the word 'TAR' with the Regular Expression box checked, the app highlights all the occurrences of 'TAA' and 'TAG' in the sequence since $R = [AG]$.

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 Viewer** 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 Viewer** 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.
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.

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 Viewer** adds a tab at the bottom for the new sequence while leaving the original sequence open.
In the left pane, click **Full Translation.** Select **Display > Amino Acid Residue Display > One Letter Code.**

The **Sequence Viewer** displays the amino acid sequence below the nucleotide sequence.
Exploring a Nucleotide Sequence Using the Sequence Viewer App
Closing the Sequence Viewer

Close the **Sequence Viewer** from the MATLAB command line using the following syntax:

```matlab
seqviewer('close')
```
Explore a Protein Sequence Using the Sequence Viewer App

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Overview of the Sequence Viewer

The **Sequence Viewer** integrates many of the sequence functions in the Bioinformatics Toolbox toolbox. Instead of entering commands in the MATLAB Command Window, you can select and enter options using the app.

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 **Sequence Viewer** accesses the NCBI database on the Web and loads amino acid sequence information for the accession number you entered.
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**
- Acidic — Red
- Basic — Light Blue
- Neutral — Black

**Function**
- Acidic — Red
- Basic — Light Blue
- Hydrophobic, nonpolar — Black
- Polar, uncharged — Green

**Hydrophobicity**
- Hydrophilic — Light Blue
- Hydrophobic — Black
<table>
<thead>
<tr>
<th>Amino Acid Color Scheme</th>
<th>Color Legend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>• Ambivalent — Dark Green</td>
</tr>
<tr>
<td></td>
<td>• External — Light Blue</td>
</tr>
<tr>
<td></td>
<td>• Internal — Orange</td>
</tr>
<tr>
<td>Taylor</td>
<td>Each amino acid is assigned its own color, based</td>
</tr>
<tr>
<td></td>
<td>on the colors proposed by W.R. Taylor on page 3-39.</td>
</tr>
</tbody>
</table>

**Closing the Sequence Viewer**

Close the **Sequence Viewer** from the MATLAB command line using the following syntax:

```matlab
seqviewer('close')
```

**References**

Compare Sequences Using Sequence Alignment Algorithms

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</tbody>
</table>

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.

Find a Model Organism to Study

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

```matlab
web('http://www.ncbi.nlm.nih.gov/books/NBK22250/')
```

The MATLAB Help browser opens with the Tay-Sachs disease page in the Genes and Diseases section of the NCBI web site. This section provides a comprehensive introduction to medical genetics. In particular, this page contains an introduction and
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.
Retrieve 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 Widow, type
   ```matlab
   ```
   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.

   ![Screenshot of NCBI search results](image)

   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.
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__000520' returns the wrong entry.

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

```matlab
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]
```

**Search 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

```matlab
```

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

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

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

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

```matlab
mouseHEXA =
```

```matlab
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]
```
Locate 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.
Locate open reading frames (ORFs) in the mouse gene. Type:

```python
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 gctgctggagggagcagctgcccgtgggcatgccccgtgctgggttccgctggtgcgt
000065 tggcgcgcgcgttgcgtttctttgcccagctgtggcgcctgcaccgcagtacacaccagcta
000129 ccacccgctacacctgtaccaccaacacacctttcaaggtcagctctctgtgcaccgcagc
000183 caagggcgggtgtctcgcagaggtttcttcagcagctacctgcattaatttcggtgagtgc
000237 gtcctggcggcgcagctttacaacgctcaaatgacagcagtccaggcctacacacacicccgc
000291 ctcgcacagctcaatgtaatattcacccactctgtgcacagctccaccgacccgaggagc
000345 aggctcagctttactgtgcacagctaccacacacacacctgggcacacacacaccaccgagc
000400 tggcgcgcgcgttgcgtttctttgcccagctgtggcgcctgcaccgcagtacacaccagcta
000455 ccacccgctacacctgtaccaccaacacacctttcaaggtcagctctctgtgcaccgcagc
000510 caagggcgggtgtctcgcagaggtttcttcagcagctacctgcattaatttcggtgagtgc
000565 gtcctggcggcgcagctttacaacgctcaaatgacagcagtccaggcctacacacacicccgc
000620 ctcgcacagctcaatgtaatattcacccactctgtgcacagctccaccgacccgaggagc
000675 aggctcagctttactgtgcacagctaccacacacacacctgggcacacacacaccaccgagc
000730 tggcgcgcgcgttgcgtttctttgcccagctgtggcgcctgcaccgcagtacacaccagcta
000785 ccacccgctacacctgtaccaccaacacacctttcaaggtcagctctctgtgcaccgcagc
000840 caagggcgggtgtctcgcagaggtttcttcagcagctacctgcattaatttcggtgagtgc
000895 gtcctggcggcgcagctttacaacgctcaaatgacagcagtccaggcctacacacacicccgc
000950 ctcgcacagctcaatgtaatattcacccactctgtgcacagctccaccgacccgaggagc
001005 aggctcagctttactgtgcacagctaccacacacacacctgggcacacacacaccaccgagc
001060 tggcgcgcgcgttgcgtttctttgcccagctgtggcgcctgcaccgcagtacacaccagcta
001115 ccacccgctacacctgtaccaccaacacacctttcaaggtcagctctctgtgcaccgcagc
001170 caagggcgggtgtctcgcagaggtttcttcagcagctacctgcattaatttcggtgagtgc
001225 gtcctggcggcgcagctttacaacgctcaaatgacagcagtccaggcctacacacacicccgc
001280 ctcgcacagctcaatgtaatattcacccactctgtgcacagctccaccgacccgaggagc
001335 aggctcagctttactgtgcacagctaccacacacacacctgggcacacacacaccaccgagc
001390 tggcgcgcgcgttgcgtttctttgcccagctgtggcgcctgcaccgcagtacacaccagcta
001445 ccacccgctacacctgtaccaccaacacacctttcaaggtcagctctctgtgcaccgcagc
001500 caagggcgggtgtctcgcagaggtttcttcagcagctacctgcattaatttcggtgagtgc
001555 gtcctggcggcgcagctttacaacgctcaaatgacagcagtccaggcctacacacacicccgc
001610 ctcgcacagctcaatgtaatattcacccactctgtgcacagctccaccgacccgaggagc
001665 aggctcagctttactgtgcacagctaccacacacacacctgggcacacacacaccaccgagc
001720 tggcgcgcgcgttgcgtttctttgcccagctgtggcgcctgcaccgcagtacacaccagcta
001775 ccacccgctacacctgtaccaccaacacacctttcaaggtcagctctctgtgcaccgcagc
001830 caagggcgggtgtctcgcagaggtttcttcagcagctacctgcattaatttcggtgagtgc
001885 gtcctggcggcgcagctttacaacgctcaaatgacagcagtccaggcctacacacacicccgc
001940 ctcgcacagctcaatgtaatattcacccactctgtgcacagctccaccgacccgaggagc
001995 aggctcagctttactgtgcacagctaccacacacacacctgggcacacacacaccaccgagc
002050 tggcgcgcgcgttgcgtttctttgcccagctgtggcgcctgcaccgcagtacacaccagcta
002105 ccacccgctacacctgtaccaccaacacacctttcaaggtcagctctctgtgcaccgcagc
002160 caagggcgggtgtctcgcagaggtttcttcagcagctacctgcattaatttcggtgagtgc
002215 gtcctggcggcgcagctttacaacgctcaaatgacagcagtccaggcctacacacacicccgc
002270 ctcgcacagctcaatgtaatattcacccactctgtgcacagctccaccgacccgaggagc
002325 aggctcagctttactgtgcacagctaccacacacacacctgggcacacacacaccaccgagc
002380 tggcgcgcgcgttgcgtttctttgcccagctgtggcgcctgcaccgcagtacacaccagcta
002435 ccacccgctacacctgtaccaccaacacacctttcaaggtcagctctctgtgcaccgcagc
002490 caagggcgggtgtctcgcagaggtttcttcagcagctacctgcattaatttcggtgagtgc
002545 gtcctggcggcgcagctttacaacgctcaaatgacagcagtccaggcctacacacacicccgc
002600 ctcgcacagctcaatgtaatattcacccactctgtgcacagctccaccgacccgaggagc
002655 aggctcagctttactgtgcacagctaccacacacacacctgggcacacacacaccaccgagc
002710 tggcgcgcgcgttgcgtttctttgcccagctgtggcgcctgcaccgcagtacacaccagcta
002765 ccacccgctacacctgtaccaccaacacacctttcaaggtcagctctctgtgcaccgcagc
002820 caagggcgggtgtctcgcagaggtttcttcagcagctacctgcattaatttcggtgagtgc
002875 gtcctggcggcgcagctttacaacgctcaaatgacagcagtccaggcctacacacacicccgc
002930 ctcgcacagctcaatgtaatattcacccactctgtgcacagctccaccgacccgaggagc
002985 aggctcagctttactgtgcacagctaccacacacacacctgggcacacacacaccaccgagc
003040 tggcgcgcgcgttgcgtttctttgcccagctgtggcgcctgcaccgcagtacacaccagcta
003095 ccacccgctacacctgtaccaccaacacacctttcaaggtcagctctctgtgcaccgcagc
003150 caagggcgggtgtctcgcagaggtttcttcagcagctacctgcattaatttcggtgagtgc
003205 gtcctggcggcgcagctttacaacgctcaaatgacagcagtccaggcctacacacacicccgc
003260 ctcgcacagctcaatgtaatattcacccactctgtgcacagctccaccgacccgaggagc
003315 aggctcagctttactgtgcacagctaccacacacacacctgggcacacacacaccaccgagc
003370 tggcgcgcgcgttgcgtttctttgcccagctgtggcgcctgcaccgcagtacacaccagcta
003425 ccacccgctacacctgtaccaccaacacacctttcaaggtcagctctctgtgcaccgcagc
003480 caagggcgggtgtctcgcagaggtttcttcagcagctacctgcattaatttcggtgagtgc
003535 gtcctggcggcgcagctttacaacgctcaaatgacagcagtccaggcctacacacacicccgc
003590 ctcgcacagctcaatgtaatattcacccactctgtgcacagctccaccgacccgaggagc
003645 aggctcagctttactgtgcacagctaccacacacacacctgggcacacacacaccaccgagc
```

### Compare 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

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

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

```matlab
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.
Globally align the two amino acid sequences, using the Needleman-Wunsch algorithm. Type

```
[GlobalScore, GlobalAlignment] = nwalign(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%.
Compare Sequences Using Sequence Alignment Algorithms

Identities = 491/812 (60%), Positives = 575/812 (71%)

260

3-51
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.

```plaintext
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.

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

humanProteinORF =

MTSSRLWFLLLLAAAFAGRATALWpPQNFQTSQRYVLYPNNFQFQYDV
SSAAAQPSCVLDEAFQRYRDLLFGSWSWPRLTGTKHTLEKNVLVSVV
TPGCNQLPTESEVENYTLTINDQCLLLLSETVWALRGLETFSQLVWKA
EGTFFINKTEIEDFPRFPHRGLLLDTSRHYPLSILDSTDVMAYNKLNV
FHWHYVDDPSFYPESFFTPELMRKGSYNVPVTIHHTAQVDKVKEVIEYARLRG
IRVLAEDFTPGHTLWGPISGIPGTTPCYSGSEPSGTFGVNPWMLNTYEF
MSTTFLEVSVPFPDFLYLHGLGDEVDFTCWKSNSPEIQDFMRRKKGFGEDFKQ
LESFYIQTLDDLIVSYYKGYVWVFEVDFDNKVIQPDTIIVQWREIPVNY
MKELELVTKAGFRALLSAAPWYLNRIISYGPDKDFYIVTEPFAEGTPEQKA
LVVGEACMWGEYVDNTNLVPRLVPRAGAVAERLWSNKLTSDLTFAYERL
SHFRC3EELLRRGVQAQPLNVGFCEQFEQT*
```
mouseProteinORF = mouseProtein(11:mouseStops(1))

mouseProteinORF =

MAGCRLWVSLLAALACLATALWPPQYIQTYHRRYTLYPNNFQFRYHV
SSAAQAGCVLDEFARRYRNLLFGSWSWPRPSFNKQQTILKTNILVVSVV
TAECLNEPNLESVENYLTINDQCLLASSETVWGALRGLYTFSQLLVWKSA
EGTFFINKTOKDFPRFPHRGVLLLDTSRHYLPSSILDLDVMAYNKFNV
FHWHLVDDSSFPYESFTFPELTRKGSFNPVTHIYTAQDVEVIEYARLRG
IRVLAEDTPGHTLSWGPAPGLLTPCYSGSHLSGTGFPVNPSSLNSYDF
MSTLFLEISSVFPDFYLHGDEVDFTCWKSNPIQAMKKGFTDFKQL
ESFYIQTLLDIVSDYDKGYVYVVFVFDNKVVRPDTIIQVWREEMPVEYM
LEMQDITRAGFRALLSAWPWLYNKYPDKMDYKVEPLAFHGTEPEKAL
VIGGEACMWGEYVDDSLVPRLWPRAGAVAERLWSSNLTIDFAKRLS
HFRCELVRRGIAQPISVGCCEQEFEQT*

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.
Another way to truncate an amino acid sequence to only those amino acids in the protein is to first truncate the nucleotide sequence with indices from 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] = nwalign(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-AMTSSRLWSSLAAAFAAGRATALWPQPQNFQTSQDQRYV . . .
   || | ||:: ||: ||:: ||: ||:: ||: ||: . . .
RGAGRWAMAGCRLWVSLLLAAALACLALTALWPQYIQTYHRRYT . . .

Show the alignment in color.

showalignment(LocalAlignment)
Compare Sequences Using Sequence Alignment Algorithms

Identities = 454/547 (83%), Positives = 514/547 (94%)

1
RGDQR-AMTSRNLWFSLLLAAAFACRATALWPUPOQNTSDQ RyVLPNNFQFGQVDVSSAAQPG

1
RGAGRUMAGCRCRVSSLAAAACLATALWUPUPYIQTHRRYTLYPNNFQFRYVHSVSSAAQAG

64
CSVLDFAFQRGRDLDLFGDSWPRPFLGTGRHTLEKVNLVVSVVTGGCNQPLTESVENYTLINT

65
CVVLDEAFRRYRNLDFGSGSWPRPSFSNKQQTLLGKNIVALVSVVTACECEFPRNFLESVENYTLINT

128
DDQCLLLSETVWGLRGEQTFSLQWKSAGETTFINKTEIEDFPRFHRGGLLDSRHYLPLSS

129
DDQCLLASETVWGLRGEQTFSLQWKSAGETTFINKTKIKDFPRFHRGVLGLSRSRHYLPLSS

192
ILDLDVMAYNKVLNFVHVLVDDLSPFPYESFTFPFELMRKGSYNPVTHIYTAQDVKEVIEYARLR

193
ILDLDVMAYNKLFNHVHLVDDLSPFPYESFTFPFELTRKGSFNPVTHIYTAQDVKEVIEYARLR

256
GIRVLAEDFTGHTLSWPGIPGGLTPCSGSEPSGTGFPGVNPSPLNNTYEFMSTFHEVSSVFP

257
GIRVLAEDFTGHTLSWPGAPGGLTPCSGSHLSGTGFPGVNPSPLNSTDFMSTLEISSVFP

320
DFYLHGGDEVDFTCWKSNPEIQDFMRKKGFGEDFKQLESFYIQTLDDIVSSYKGYVWQEFV

321
DFYLHGGDEVDFTCWKSNPIQAFMKKKGFG-TDFKQLESFYIQTLDDIVSYDKGYYVWQEFV

384
DNKVKIQPDTIIQWREDIPVNYKELELVTAGFRRALLSAPWLYNRIYGPWDKFDYVVEPLA

384
DNKVKVPDTIIQWREEMPVEYMLEMQDITRAGFRRALLSAPWLYNRVLYGPWDKMYKVEPLA

448
FEQTEQKLVGGEACMUGSEYVDPNTNLVPRLWPRAGAVAEELWSNKLTSDLTFAYERLSSHRC

448
FHTQEQQVLGGEACMUGSEYVDSTNLVPRLWPRAGAVAEELWSNLTIDFAFKRLSSHRC

512
ELLRRGVAQQPLNVGFCEQFEQT*APGETEGAGC

512
ELVRRGIAQPISVGCEFDFQET*ATSAEHPGGC
View and Align Multiple Sequences

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Overview of the Sequence Alignment App

The **Sequence Alignment** app integrates many sequence and multiple alignment functions in the toolbox. Instead of entering commands in the MATLAB Command Window, you can use this app to visually inspect a multiple alignment and make manual adjustments.

Visualize Multiple Sequence Alignment

1. Read a multiple sequence alignment file of the gag polyprotein for several HIV strains.
   
   $$\text{gagaa} = \text{multialignread}'aagag.aln'$$

2. View the aligned sequences in the **Sequence Alignment** app.
   
   seqalignviewer(gagaa);
Adjust Sequence Alignments Manually

Algorithms for aligning multiple sequences do not always produce an optimal result. By visually inspecting the alignment, you can identify areas whose alignment can be improved by a manual adjustment.

1. To better visualize the sequence alignments, you can zoom in by selecting **Display > Zoom in**. Select this option multiple times until you achieve the zoom level you want.
2. Identify an area where you could improve the alignment.
Click a letter or a region. The selected region is the center block. You can then drag the sequence(s) to the left or right of the center block.
To move a single letter (T in this example), click and drag the letter T (center block) to the right to insert a gap.
5 Close the gap by dragging the letter back to the left.
You can also move multiple residues (a subsequence). Suppose you want to move a subsequence to available gaps. First select the gap region that you want to fill in.
Drag the subsequence(s) from the right or left of the gap region into the gap area.
Suppose you want to remove one or more of the aligned sequences. First select the sequence(s) to be removed. Then select **Edit > Delete Sequences**.
9 Remove empty columns by selecting **Edit > Remove Empty Columns**.
After the edit, you can export the aligned sequences or consensus sequence to a FASTA file or MATLAB Workspace from the **File** menu.

### Rearrange Rows

You can move the rows (sequences) up or down by one row. You can also move selected rows to the top or bottom of the list.
The selected sequence moves to the bottom of the list.
Generate Phylogenetic Tree from Aligned Sequences

You can generate a phylogenetic tree using the aligned sequences from within the app. You can select a subset of sequences or use all the sequences to generate a tree.

Select Display > View Tree > Selected... to generate a tree from selected sequences.
A phylogenetic tree for the sequences is displayed in the **Phylogenetic Tree** app. For details on the app, see “Using the Phylogenetic Tree App” on page 5-2.
See Also
NGS Browser | Sequence Alignment | Sequence Viewer | seqalignviewer
More About

- “Sequence Alignments” on page 1-10
- “Aligning Pairs of Sequences”
Microarray Analysis

- “Managing Gene Expression Data in Objects” on page 4-2
- “Representing Expression Data Values in DataMatrix Objects” on page 4-5
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- “Representing Sample and Feature Metadata in MetaData Objects” on page 4-15
- “Representing Experiment Information in a MIAME Object” on page 4-21
- “Representing All Data in an ExpressionSet Object” on page 4-25
- “Visualizing Microarray Images” on page 4-30
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 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.

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 4-5
- “Representing Expression Data Values in ExptData Objects” on page 4-11
- “Representing Sample and Feature Metadata in MetaData Objects” on page 4-15
- “Representing Experiment Information in a MIAME Object” on page 4-21
- “Representing All Data in an ExpressionSet Object” on page 4-25
Representing Expression Data Values in DataMatrix Objects

**In this section...**

“Overview of DataMatrix Objects” on page 4-5
“Constructing DataMatrix Objects” on page 4-6
“Getting and Setting Properties of a DataMatrix Object” on page 4-6
“Accessing Data in DataMatrix Objects” on page 4-7

**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.

```matlab
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.

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

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

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

4 Use the DataMatrix constructor function to create a small DataMatrix object from the gene expression data.

```matlab
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 0.981
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.

```matlab
get(dmo)
```

```
Name:
```
Use the `set` method to specify a name for the DataMatrix object, `dmo`.

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

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

```matlab
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`:

```matlab
dmo2 = dmo(1:5,2:3)
dmo2 =
   9.5   11.5
   1.699 -0.026
   0.146 -0.129
```

**Representing Expression Data Values in DataMatrix Objects**

4-7
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 =

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>9.5</th>
<th>11.5</th>
<th>13.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS DNA</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>YAL003W</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>YAL012W</td>
<td>0.157</td>
<td>0.175</td>
<td>0.467</td>
<td>-0.379</td>
</tr>
<tr>
<td>YAL026C</td>
<td>0.246</td>
<td>0.796</td>
<td>0.384</td>
<td>0.981</td>
</tr>
<tr>
<td>YAL034C</td>
<td>-0.235</td>
<td>0.487</td>
<td>-0.184</td>
<td>-0.669</td>
</tr>
</tbody>
</table>

Use dot indexing to delete an entire variable from dmo:

dmo.YAL034C = []
dmo =

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>9.5</th>
<th>11.5</th>
<th>13.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS DNA</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>YAL003W</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>YAL012W</td>
<td>0.157</td>
<td>0.175</td>
<td>0.467</td>
<td>-0.379</td>
</tr>
<tr>
<td>YAL026C</td>
<td>0.246</td>
<td>0.796</td>
<td>0.384</td>
<td>0.981</td>
</tr>
</tbody>
</table>

Use dot indexing to delete two columns from dmo:

dmo.('::')(2:3) = []
$$d_{mo} =$$

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>13.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS DNA</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>YAL003W</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>YAL012W</td>
<td>0.157</td>
<td>-0.379</td>
</tr>
<tr>
<td>YAL026C</td>
<td>0.246</td>
<td>0.981</td>
</tr>
</tbody>
</table>
Representing Expression Data Values in ExptData Objects

In this section...

“Overview of ExptData Objects” on page 4-11
“Constructing ExptData Objects” on page 4-12
“Using Properties of an ExptData Object” on page 4-12
“Using Methods of an ExptData Object” on page 4-13
“References” on page 4-14

Overview of ExptData Objects

You can use an ExptData object to store expression values from a microarray experiment. An ExprData 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):

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>100001_at</td>
<td>2.26</td>
<td>20.14</td>
<td>31.66</td>
</tr>
<tr>
<td>100002_at</td>
<td>158.86</td>
<td>236.25</td>
<td>206.27</td>
</tr>
<tr>
<td>100003_at</td>
<td>68.11</td>
<td>105.45</td>
<td>82.92</td>
</tr>
<tr>
<td>100004_at</td>
<td>74.32</td>
<td>96.68</td>
<td>84.87</td>
</tr>
<tr>
<td>100005_at</td>
<td>75.05</td>
<td>53.17</td>
<td>57.94</td>
</tr>
<tr>
<td>100006_at</td>
<td>80.36</td>
<td>42.89</td>
<td>77.21</td>
</tr>
<tr>
<td>100007_at</td>
<td>216.64</td>
<td>191.32</td>
<td>219.48</td>
</tr>
</tbody>
</table>

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

Representing Sample and Feature Metadata in MetaData Objects

In this section...

“Overview of MetaData Objects” on page 4-15
“Constructing MetaData Objects” on page 4-16
“Using Properties of a MetaData Object” on page 4-19
“Using Methods of a MetaData Object” on page 4-19

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:
<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Type</th>
<th>Strain</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8</td>
<td>'Male'</td>
<td>'Wild type'</td>
<td>'amygdala'</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>'Male'</td>
<td>'Wild type'</td>
<td>'amygdala'</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>'Male'</td>
<td>'Wild type'</td>
<td>'amygdala'</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>'Male'</td>
<td>'A/J'</td>
<td>'amygdala'</td>
</tr>
<tr>
<td>E</td>
<td>8</td>
<td>'Male'</td>
<td>'A/J'</td>
<td>'amygdala'</td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td>'Male'</td>
<td>'C57BL/6J'</td>
<td>'amygdala'</td>
</tr>
</tbody>
</table>

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.

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

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

   ```python
   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.
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.data package 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

4-17
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

<table>
<thead>
<tr>
<th>ID</th>
<th>Gender</th>
<th>Age</th>
<th>Type</th>
<th>Strain</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>129S6/SvEvTac</td>
<td>amygdala</td>
</tr>
<tr>
<td>B</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>129S6/SvEvTac</td>
<td>amygdala</td>
</tr>
<tr>
<td>C</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>129S6/SvEvTac</td>
<td>amygdala</td>
</tr>
<tr>
<td>D</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>A/J</td>
<td>amygdala</td>
</tr>
<tr>
<td>E</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>A/J</td>
<td>amygdala</td>
</tr>
<tr>
<td>F</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>C57BL/6J</td>
<td>amygdala</td>
</tr>
<tr>
<td>G</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>C57BL/6J</td>
<td>amygdala</td>
</tr>
<tr>
<td>H</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>129S6/SvEvTac</td>
<td>cingulate cortex</td>
</tr>
<tr>
<td>I</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>129S6/SvEvTac</td>
<td>cingulate cortex</td>
</tr>
<tr>
<td>J</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>A/J</td>
<td>cingulate cortex</td>
</tr>
<tr>
<td>K</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>A/J</td>
<td>cingulate cortex</td>
</tr>
<tr>
<td>L</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>A/J</td>
<td>cingulate cortex</td>
</tr>
<tr>
<td>M</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>C57BL/6J</td>
<td>cingulate cortex</td>
</tr>
<tr>
<td>N</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>C57BL/6J</td>
<td>cingulate cortex</td>
</tr>
<tr>
<td>O</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>129S6/SvEvTac</td>
<td>hippocampus</td>
</tr>
<tr>
<td>P</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>129S6/SvEvTac</td>
<td>hippocampus</td>
</tr>
<tr>
<td>Q</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>A/J</td>
<td>hippocampus</td>
</tr>
<tr>
<td>R</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>A/J</td>
<td>hippocampus</td>
</tr>
<tr>
<td>S</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>C57BL/6J</td>
<td>hippocampus</td>
</tr>
<tr>
<td>T</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>C57BL/6J4</td>
<td>hippocampus</td>
</tr>
<tr>
<td>U</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>129S6/SvEvTac</td>
<td>hypothalamus</td>
</tr>
<tr>
<td>V</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>129S6/SvEvTac</td>
<td>hypothalamus</td>
</tr>
<tr>
<td>W</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>A/J</td>
<td>hypothalamus</td>
</tr>
<tr>
<td>X</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>A/J</td>
<td>hypothalamus</td>
</tr>
<tr>
<td>Y</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>C57BL/6J</td>
<td>hypothalamus</td>
</tr>
<tr>
<td>Z</td>
<td>Male</td>
<td>8</td>
<td>Wild type</td>
<td>C57BL/6J</td>
<td>hypothalamus</td>
</tr>
</tbody>
</table>

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

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

Sample Names:  
A, B, ..., Z (26 total)

Variable Names and Meta Information:

```python
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:

```matlab
objectname.propertyname
```

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

```matlab
MDObj2.NVariables
```

```matlab
ans =
    5
```

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

```matlab
objectname.propertyname = propertyvalue
```

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

```matlab
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:

```matlab
objectname.methodname
```

or

```matlab
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

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

   geoStruct =
   
   ```matlab
   Header: [1x1 struct]
   Data: [12488x12 bioma.data.DataMatrix]
   ```

3. Use the MIAME constructor function to create a MIAME object from the structure.
   
   ```python
   MIAMEObj1 = MIAME(geoStruct);
   ```

4. Display information about the MIAME object, MIAMEObj1.
   
   ```python
   MIAMEObj1
   ```

   MIAMEObj1 =
   
   Experiment Description:
   
   Author name: Mika, Silvennoinen
   Riikka, Kivelä
   Maarit, Lehti
   Anna-Maria, Touvras
   Jyrki, Komulainen
   Veikko, Vihko
   Heikki, Bainulainen
   
   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.
   
   ```python
   import bioma.data.*
   ```

2. Use the MIAME constructor function to create a MIAME object using individual properties.
   
   ```python
   ```
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 in an ExpressionSet Object

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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 4-11
- “Representing Sample and Feature Metadata in MetaData Objects” on page 4-15
- “Representing Experiment Information in a MIAME Object” on page 4-21

1. Import the bioma package so that the ExpressionSet constructor function is available.

   ```
   import bioma.*
   ```

2. Construct an ExpressionSet object from E0bj, 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
   ```
Using Properties of an ExpressionSet Object

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

\[ \text{objectname.propertyname} \]

For example, to determine the number of samples in an ExpressionSet object:

\[
\text{ESObj.NSamples}
\]

\[
\text{ans} =
\]

\[
26
\]

**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:

\[ \text{objectname.methodname} \]

or

\[ \text{methodname(objectname)} \]

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

\[
\text{ESObj.sampleVarNames}
\]

\[
\text{ans} =
\]

\[
\text{'Gender' 'Age' 'Type' 'Strain' 'Source'}
\]

To retrieve the experiment information contained in an ExpressionSet object:
exptInfo(ESObj)

ans =

Experiment description
   Author name: Mika,Silvennoinen
   Riikka,Kivelä
   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.
Overview of the Mouse Example


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/

The microarray data is also available on the Gene Expression Omnibus Web site at


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 4-30.

1 Read data from a file into a MATLAB structure. For example, in the MATLAB Command Window, type

```matlab
pd = gprread('mouse_alpd.gpr')
```

Information about the structure displays in the MATLAB Command Window:

```matlab
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

```matlab
pd.ColumnNames
```

The column names are shown below.

```plaintext
ans =
    'X'
    'Y'
    'Dia.'
    'F635 Median'
    'F635 Mean'
    'F635 SD'
    'B635 Median'
    'B635 Mean'
    'B635 SD'
    '% > B635+1SD'
    '% > B635+2SD'
    'F635 % Sat.'
```
Access the names of the genes. For example, to list the first 20 gene names, type

```matlab
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'
```
Spatial Images of Microarray Data

This procedure illustrates how to visualize microarray data by plotting image maps. The function `maimage` 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, `maimage` 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 4-31.

1. Plot the median values for the red channel. For example, to plot data from the field `F635 Median`, type

   ```matlab
   figure
   maimage(pd,'F635 Median')
   ```

   The MATLAB software plots an image showing the median pixel values for the foreground of the red (Cy5) channel.
Plot the median values for the green channel. For example, to plot data from the field `F532 Median`, type

```matlab
figure
maimage(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.

```matlab
figure
maimage(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.
Plot the medial values for the green background. The field B532 Median shows the median values for the background of the green channel.

```matlab
figure
maimage(pd,'B532 Median')
```

The MATLAB software plots an image for the background of the green channel.
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}
```
Use the function `maimage` to show pseudocolor images of the foreground and background. You can use the function `subplot` to put all the plots onto one figure.

```matlab
figure
subplot(2,2,1);
maimage(wt,'F635 Median')
subplot(2,2,2);
maimage(wt,'F532 Median')
subplot(2,2,3);
maimage(wt,'B635 Median')
subplot(2,2,4);
maimage(wt,'B532 Median')
```

The MATLAB software plots the images.

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 colormap editor function.

colormap hot

The MATLAB software plots the images.

The function maimage 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;
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

```matlab
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')
```
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

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.

```matlab
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.
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)`.

```matlab
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.
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.

Normalize the points to evenly distribute them on either side of the line. Use the function `manorm` to perform global mean normalization.

```matlab
normcy5 = manorm(cy5Data);
normcy3 = manorm(cy3Data);
```

If you plot the normalized data you will see that the points are more evenly distributed about the $y = x$ line.

```matlab
figure
maloglog(normcy5,normcy3,'labels',wt.IDs(~badPoints),...
    'factorlines',2)
xlabel('F635 Median - B635 (Control)');
ylabel('F532 Median - B532 (Voxel A1)');
```
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`.

```matlab
figure
mairplot(normcy5,normcy3,'labels',wt.IDs(~badPoints),...
       'factorlines',2)
```

The MATLAB software plots the image.
You can click the points in this plot to see the name of the gene associated with the plot.
Phylogenetic Analysis
Using the Phylogenetic Tree App

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Overview of the Phylogenetic Tree App

The Phylogenetic Tree app allows you to view, edit, format, and explore phylogenetic tree data. With this app you can prune, reorder, rename branches, and explore distances. You can also open or save Newick or ClustalW tree formatted files. The following sections give a description of menu commands and features for creating publishable tree figures.

Opening the Phylogenetic Tree App

This section illustrates how to draw a phylogenetic tree from data in a phytree object or a previously saved file.

The Phylogenetic Tree app 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

   \[ \text{tr} = \text{phytreeread('pf00002.tree')} \]

   The MATLAB software creates a phytree object.

   Phylogenetic tree object with 33 leaves (32 branches)

2. View the phylogenetic tree using the app.
phytreeviewer(tr)

Alternatively, click **Phylogenetic Tree** on the **Apps** tab.

### 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 Viewer Command

Use the New Viewer command to open tree data from a file into a second Phylogenetic Tree viewer.

1. From the File menu, select New Viewer.

   The Open A Phylogenetic Tree dialog box opens.
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 viewer 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 the app.

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 app uses the file extension `.tree` for Newick-formatted files, but you can use any Newick-formatted file with any extension.

   The app 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 the data using the app.

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 app replaces the current tree data with data from the selected object.

**Open Original in New Viewer**

There may be times when you make changes that you would like to undo. The `Phylogenetic Tree` app does not have an undo command, but you can get back to the original tree you started viewing with the **Open Original in New Viewer** command.

From the **File** menu, select **Open Original in New Viewer**.

A new Phylogenetic Tree viewer 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**.

The app 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 Viewer Command**

Because some of the Phylogenetic Tree viewer 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 Viewer**, and then select either **With Hidden Nodes** or **Only Displayed**.

A new Phylogenetic Tree viewer opens with a copy of the tree.

2 Use the new figure to continue your analysis.

**Export to Workspace Command**

The **Phylogenetic Tree** app 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 app 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 app.

1 From the **File** menu, select **Print to Figure**, and then select either **With Hidden Nodes** or **Only Displayed**.

The **Print Phylogenetic Tree to Figure** dialog box opens.
2 Select one of the **Rendering Types**.
<table>
<thead>
<tr>
<th>Rendering Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>'square' (default)</td>
<td>![Phylogenetic Tree Diagram]</td>
</tr>
<tr>
<td>Rendering Type</td>
<td>Description</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
</tr>
<tr>
<td>'angular'</td>
<td><img src="image1" alt="Angular Rendering" /></td>
</tr>
<tr>
<td>'radial'</td>
<td><img src="image2" alt="Radial Rendering" /></td>
</tr>
<tr>
<td>Rendering Type</td>
<td>Description</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
</tr>
<tr>
<td>'equalangle'</td>
<td><img src="image1.png" alt="Diagram" /> <strong>Tip</strong> This rendering type hides the significance of the root node and emphasizes clusters, thereby making it useful for visually assessing clusters and detecting outliers.</td>
</tr>
<tr>
<td>'equaldaylight'</td>
<td><img src="image2.png" alt="Diagram" /> <strong>Tip</strong> This rendering type hides the significance of the root node and emphasizes clusters, thereby making it useful for visually assessing clusters and detecting outliers.</td>
</tr>
</tbody>
</table>
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.

**Print Preview Command**

When you print from the **Phylogenetic Tree** app or a MATLAB Figure window (with a tree published from the viewer), you can specify setup options for printing a tree.

1 From the **File** menu, select **Print Preview**.

The **Print Preview** window opens, which you can use to select page formatting options.
2 Select the page formatting options and values you want, and then click **Print**.

**Print Command**

Use the **Print** command to make a copy of your phylogenetic tree after you use the **Print Preview** 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:

![Tools Menu](image)

**Inspect Mode**

Viewing a phylogenetic tree in the **Phylogenetic Tree** app 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 app 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 app 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.

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**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 Brand Mode** icon.

The app 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 app 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 app 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** app takes the node names from a 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 app is set to rename mode.

2 Click a branch or leaf node.

   ![Branch and Leaf Nodes](image)

A text box opens with the current name of the node.

3 In the text box, edit or enter a new name.

   ![Text Box with New Name](image)

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.

   ![Prune Mode Icon](image)
The app 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 app 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 app 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 app.

**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 Phylogenetic Tree app reference.

Use the Help menu to select quick links to the Bioinformatics Toolbox documentation for phylogenetic analysis functions, tutorials, and the phytreeviewer reference.