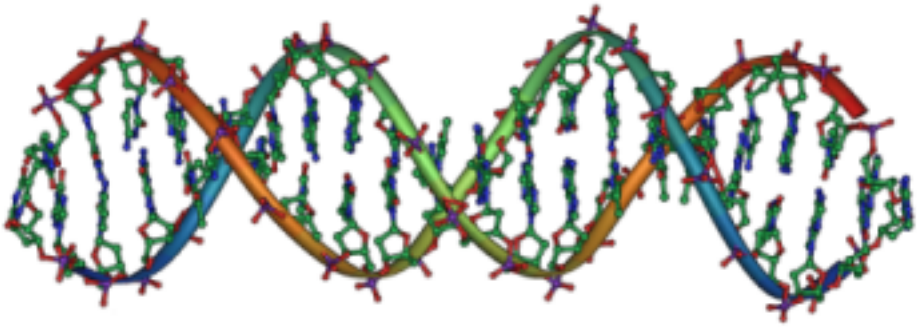
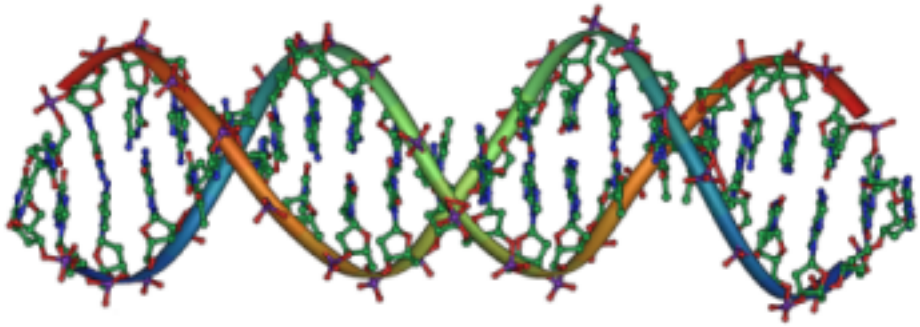
Bio Users Guide



6.2 Edition

Bio Users Guide :



6.2 Edition
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Preface

Bio-Informatics is the use of techniques from applied mathematics, informatics, statistics, and computer science to solve biological problems. Major research efforts in the field include sequence alignment, gene finding, genome assembly, protein structure alignment, protein structure prediction, prediction of gene expression and protein-protein interactions, and the modeling of evolution.

To address the requirements of these efforts, a wide spectrum of bio-informatics tools are available. These tools, while powerful, are packaged according to the individual tastes of the developers.

The Bio-informatics Roll is a collection of some of the most common bio-informatics tools that are being used by the community today. This roll is being developed in an attempt to standardize and ease packaging and installation of these tools.

Chapter 1. Overview

Table 1-1. Summary

Name	bio
Version	6.2
Maintained By	Rocks Group
Architecture	i386, x86_64
Compatible with Rocks®	6.2

The bio roll has the following requirements of other rolls. Compatability with all known rolls is assured, and all known conflicts are listed. There is no assurance of compatiblity with third-party rolls.

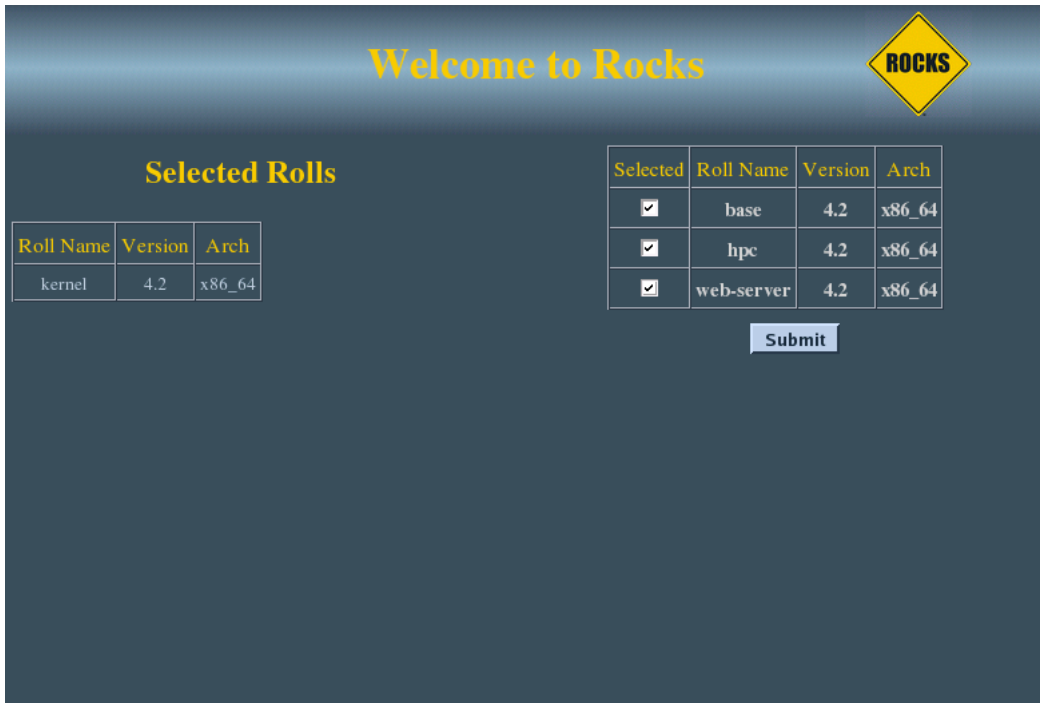
Table 1-2. Compatibility

Requires	Conflicts
Base	
HPC	
Kernel	
OS	
Web Server	

Chapter 2. Installing

2.1. On a New Server

The bio roll should be installed during the initial installation of your server (or cluster). This procedure is documented in section 3.2 of the Rocks® usersguide. You should select the bio roll from the list of available rolls when you see a screen that is similar to the one below.



2.2. On an Existing Server

The bio Roll may also be added onto an existing server (or frontend). For sake of discussion, assume that you have an iso image of the roll called `bio.iso`. The following procedure will install the Roll, and after the server reboots the Roll should be fully installed and configured.

```
$ su - root
# rocks add roll bio.iso
# rocks enable roll bio
# cd /export/rocks/install
# rocks create distro
# rocks run roll bio | bash
# init 6
```

Chapter 3. Using

3.1. List of packages present in the Bio Roll

The Bio Roll contains a suite of Bio-informatics applications, most commonly in use by the bio-informatics community. The list of applications is as follows:

- HMMER - http://hmmer.janelia.org/
- NCBI BLAST - From National Center for Biotechnology Information - www.ncbi.nlm.nih.gov/BLAST/[2]
- MpiBLAST - From Los Alamos National Laboratory - http://mpiblast.lanl.gov/
- biopython - www.biopython.org
- ClustalW - From the European BioInformatics Institute - http://www.ebi.ac.uk/clustalw/
- MrBayes - From School of Computational Science at the Florida State University - http://mrbayes.csit.fsu.edu/
- T_Coffee - From Information Genomique et Structurale at Centre National de la Recherche Scientifique - The T-Coffee Home Page[7]
- Emboss - From European Molecular Biology Institute - http://emboss.sourceforge.net/
- Phylip - From the Dept. of Biology at the University of Washington - http://evolution.genetics.washington.edu/phylip.html
- fasta - From the University of Virginia - http://fasta.bioch.virginia.edu/
- Glimmer - From Center for Bioinformatics and Computational Biology at the University of Maryland - http://www.cbcb.umd.edu/software/glimmer/
- TIGR Assembler - From the J. Craig Venter Institute - http://www.jcvi.org/cms/research/software/
- All the perl utilities mentioned below are from CPAN
- perl-bioperl
- perl-bioperl-ext
- perl-bioperl-run
- perl-bioperl-db

All the packages that appear below are dependencies and are already present in the base and OS Rolls. They are installed automatically during system installation.

foundation-python	flex	readline-devel
foundation-python-extras	xorg-x11-devel	gd
ReportLab	readline	gd-devel

3.2. HMMER

3.2.1. About

HMMER is an implementation of profile HMM methods for sensitive database searches using multiple sequence

alignments as queries.

The version of HMMER that is distributed with this version of Rocks was obtained from here[11]. The version as of code freeze is v2.3.2 and is distributed under the GNU General Public License v2.0.

3.2.2. Usage

HMMER is setup in the /opt/bio/hmmer directory. The HMMER execution environment is setup automatically by the login scripts. The environment contains HMMER_DB variable which points to the directory containing the hmmer databases. By default, this is set to $HOME/bio/hmmer/db/.

HMMER has many modes of execution. For a description of all the executables that come with HMMER, please refer to the current HMMER online userguide[12]. This guide is also available on your rocks installation at /opt/bio/hmmer/Userguide.pdf

There is also a tutorial available on your cluster at /opt/bio/hmmer/tutorial/ . The description of how to use the tutorial is given in the Userguide.pdf file.

3.3. NCBI BLAST

3.3.1. About

BLAST, or Basic Local Alignment Search Tool, is a collection of tools that are used to search for and find regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases, and calculates the statistical significance of the matches. This software suite has been released free to the public by the National Centre for Biotechnology Information.

3.3.2. Usage

BLAST can be used for protein-protein comparisons or nucleotide-nucleotide comparisons. Before an example of the usage is presented, we must first define some environmental variables.

- $BLASTDB - This is the variable which points to the Blast Database. This is set to $HOME/bio/ncbi/db/. This directory should contain the databases that you would want to search. BLAST, by default, checks this location and the current working directory for the presence of the databases. This variable is set during login by system login scripts , and may be changed by the user to point to her preferred location in her startup scripts.
- $BLASTMAT - This variable points to the location where the BLAST scoring matricies are present. It is set to /opt/bio/ncbi/data. Again, they may be changed to point to a desired location on a per-user basis.

BLAST requires the presence of 2 datasets. One dataset is the input sequence that you want to search for, and the other dataset is the database that you want to search against.

Use the following procedure to run blast

- Download a BLAST database that you want to run the comparison against. The databases can be obtained from the NCBI ftp site at ftp://ftp.ncbi.nlm.nih.gov/blast/db/.

The databases available on the site mentioned above are pre-formatted.

It is recommended that the blast databases be stored at the $BLASTDB location.

Visit ftp://ftp.ncbi.nlm.nih.gov/blast/db/ in your browser to see a list of available preformatted databases.

Download one of these on to your cluster using wget.

```
[nostromo@xxx ~]$ wget -q ftp://ftp.ncbi.nlm.nih.gov/blast/db/nt.08.tar.gz
[nostromo@xxx ~]$ gunzip -c nt.08.tar.gz | ( cd $BLASTDB/ && tar -xf -)
```

- The above method downloads a formatted database, and untars it into $BLASTDB.

  Unformatted databases can be obtained in FASTA format at ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/[15].

  Visit ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/[16] in your web browser

If you've downloaded the databases from ftp://ftp.ncbi.nlm.nih.gov/blast/db/, then DO NOT run formatdb.

Run the formatdb command to format the database to the BLAST format. For this example, we'll use the Drosophila Melanogaster (fruitfly) nucleotide database

```
[nostromo@xxx ~]$ cd $BLASTDB
[nostromo@xxx ~]$ wget -q ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/drosoph.nt.gz
[nostromo@xxx ~]$ gunzip drosoph.nt.gz
[nostromo@xxx ~]$ formatdb -p F -V T -i drosoph.nt
[nostromo@xxx ~]$ ls drosoph.nt*
drosoph.nt  drosoph.nt.nhr  drosoph.nt.nin  drosoph.nt.nsq
[nostromo@xxx ~]$ cd $HOME
```

- After the database is formatted, create a test input file.

```
[nostromo@xxx ~]$ cat > test.txt
>Test
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGC
TTCTGAACTGGTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAA
TATAGGCATAGCGCACAGACAGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACC
ATTACCACCACCATCACCATTACCACAGGTAACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAG
CCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGGTAACGAGGTAACAACCATGCGAGTGTTGAA
GTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCGATATTCTGGAAAGCAATGCC
AGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGGTGGCGATGATTG
AAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT
```

- Run the blastall program on the test input against the formatted database.

```
[nostromo@xxx ~]$ blastall --help
```

gives a list of all the options that you can use to run the blastall program.

```
[nostromo@xxx ~]$  blastall -d drosoph.nt -p blastn -i test.txt
BLASTN 2.2.18 [Mar-02-2008]


Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs",  Nucleic Acids Res. 25:3389-3402.
```

```
Query= Test
         (560 letters)

Database: drosoph.nt
           1170 sequences; 122,655,632 total letters

Searching.....................................................done



                                                                  Score    E
Sequences producing significant alignments:                       (bits) Value

gi|10729531|gb|AE002936.2|AE002936 Drosophila melanogaster genom...    36   0.86
gi|10728232|gb|AE003493.2|AE003493 Drosophila melanogaster genom...    36   0.86
gi|10726497|gb|AE003698.2|AE003698 Drosophila melanogaster genom...    36   0.86
gi|10726398|gb|AE003681.2|AE003681 Drosophila melanogaster genom...    36   0.86
gi|10729308|gb|AE002665.2|AE002665 Drosophila melanogaster genom...    34   3.4
gi|10729264|gb|AE002615.2|AE002615 Drosophila melanogaster genom...    34   3.4
gi|7298233|gb|AE003648.1|AE003648 Drosophila melanogaster genomi...    34   3.4
gi|7297628|gb|AE003628.1|AE003628 Drosophila melanogaster genomi...    34   3.4
gi|10728546|gb|AE003447.2|AE003447 Drosophila melanogaster genom...    34   3.4
gi|7290819|gb|AE003441.1|AE003441 Drosophila melanogaster genomi...    34   3.4
gi|10728461|gb|AE003431.2|AE003431 Drosophila melanogaster genom...    34   3.4
gi|10728241|gb|AE003495.2|AE003495 Drosophila melanogaster genom...    34   3.4
gi|7292554|gb|AE003484.1|AE003484 Drosophila melanogaster genomi...    34   3.4
gi|10727872|gb|AE003525.2|AE003525 Drosophila melanogaster genom...    34   3.4
gi|10727399|gb|AE003587.2|AE003587 Drosophila melanogaster genom...    34   3.4
gi|10727114|gb|AE003673.2|AE003673 Drosophila melanogaster genom...    34   3.4
gi|10726705|gb|AE003740.2|AE003740 Drosophila melanogaster genom...    34   3.4
```

The above example shows how to search for the test input in a drosophila nucleotide database, and a snippet of the output file.

3.3.3. Running Blast with SGE

This section gives a very simple example of running BLAST through the provided batch system SGE.

- Create a simple submission script called blast_sge.sh containing the following -

```
#!/bin/bash
#
#$ -cwd
#$ -S /bin/bash
#$ -j y

export BLASTDB=$HOME/bio/ncbi/db/
export BLASTMAT=/opt/bio/ncbi/data/

/opt/bio/ncbi/bin/blastall -d drosoph.nt \
        -p blastn -i $HOME/test.txt      \
        -o $HOME/result.txt
```

- Run

```
[nostromo@xxx ~]$ qsub blast_sge.sh
Your job 10 ("blast_sge.sh") has been submitted
```

- The output of the Blast job is similar to the one given above and will be stored in $HOME/result.txt

3.3.4. Further Information

For further information about BLAST and its usage, please refer to the following sources

- THE NCBI Blast website - http://www.ncbi.nlm.nih.gov/BLAST/[17]
- BLAST Help page on your cluster BLAST Help Page[18]
- BLAST Program selection Guide - http://www.ncbi.nlm.nih.gov/blast/BLAST_guide.pdf[19]

3.4. ClustalW

3.4.1. About

ClustalW is a multiple sequence alignment program. The version included with this distribution is v2.0.12.

3.4.2. Using ClustalW

ClustalW can be run at the command line as

```
[nostromo@xxx ~]$ clustalw2


 ***********************************************************
 ******** CLUSTAL 2.0.12 Multiple Sequence Alignments  ********
 ***********************************************************


     1. Sequence Input From Disc
     2. Multiple Alignments
     3. Profile / Structure Alignments
     4. Phylogenetic trees

     S. Execute a system command
     H. HELP
     X. EXIT (leave program)


Your choice:
```

Choosing the option 'H' brings up the help on clustalW.

3.4.3. Further Information

Further information on the usage of ClustalW can be obtained from clustalw.doc(MS Word Document) available at /opt/bio/clustalw/doc/clustalw.doc on the frontend of your cluster.

3.5. EMBOSS

3.5.1. About

EMBOSS is the European Molecular Biology Open Software Suite, a set of tools that are used for sequence analysis by the Molecular Biology community (EMBnet).

The version of EMBOSS included with this version of Rocks is 6.1.0

3.5.2. Further Information

Information about using EMBOSS is available at http://emboss.sourceforge.net/. You may also register at their mailing list here[21].

3.6. Glimmer

3.6.1. About

Glimmer is a system for finding genes in microbial DNA, especially the genomes of bacteria, archaea, and viruses. Glimmer was developed at the Centre for BioInformatics and Computational Biology. The version that is distributed with Rocks is Glimmer v3.02.

3.6.2. Using Glimmer

Glimmer is installed at /opt/bio/glimmer/. Glimmer is run in 2 stages.

- Glimmer is trained on a particular training set of similar species to recognize genes
- Glimmer is then run on an input DNA sequence to find genes

3.6.3. Further Information

Further information about the usage of Glimmer can be found in the release notes of the software, available here[22]. This file is also available on the frontend of your cluster at /opt/bio/glimmer/glim302notes.pdf

3.7. Fasta

3.7.1. About Fasta

FASTA is a program used to search in large Protein or DNA sequence data banks. It was developed at the University of Virginia by William R. Pearson, and D.J. Lippman.

3.7.2. Usage

FASTA is installed in /opt/bio/fasta/. FASTA is run in a similar manner to NCBI Blast.

- First create a test query file

```
[nostromo@xxx ~]$ cat > test.txt
>Test
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGC
TTCTGAACTGGTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAA
TATAGGCATAGCGCACAGACAGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACC
ATTACCACCACCATCACCATTACCACAGGTAACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAG
CCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGGTAACGAGGTAACAACCATGCGAGTGTTGAA
GTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCGATATTCTGGAAAGCAATGCC
AGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGGTGGCGATGATTG
AAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT
```

- The next step is to search for this against a database sequence. For this, we can download a DNA or protein sequence database or use the ones that are provided by the program. For this example, we will use the ones present along with the fasta program in /opt/bio/fasta/.

```
[nostromo@xxx ~]$ fasta35
# fasta35
FASTA searches a protein or DNA sequence data bank
 version 35.04 Oct. 7, 2008
Please cite:
 W.R. Pearson & D.J. Lipman PNAS (1988) 85:2444-2448

 test sequence file name: test.txt
 library file name: drosoph.nt
 ktup? (1 to 6) [6]
Query: test.txt
  1>>>Test - 560 nt
Library: drosoph.nt
.......  Done!
 122655632 residues in  1170 sequences

       opt      E()
< 20     0     0:
  22     0     0:           one = represents 3 library sequences
  24     0     0:
  26     0     0:
  28     0     0:
  30     3     2:*
  32    12     9:==*=
  34    37    23:=======*=====
  36    59    48:===============*====
  38    90    79:==========================*===
```

```
  40   110   110:==================================*
  42   133   135:===========================================*
  44   147   149:================================================*
  46   151   151:=================================================*
  48   129   145:============================================     *
  50   131   132:===========================================*
  52   102   116:==================================    *
  54    92    99:================================ *
  56    80    83:============================*
  58    68    68:======================*
  60    43    55:===============   *
  62    44    44:==============*
  64    42    35:===========*==
  66    30    28:=========*
  68    25    22:=======*=
  70    20    17:=====*=
  72    18    13:====*=
  74    14    10:===*=
  76     7     8:==*
  78     7     6:=*=
  80     9     5:=*=
  82     3     4:=*
  84     0     3:*
  86     0     2:*
  88     2     2:*          inset = represents 1 library sequences
  90     1     1:*
  92     0     1:*         :*
  94     0     1:*         :*
  96     2     1:*         :*=
  98     0     0:          *
 100     0     0:          *
 102     0     0:          *
 104     0     0:          *
 106     0     0:          *
 108     0     0:          *
 110     0     0:          *
 112     0     0:          *
 114     0     0:          *
 116     0     0:          *
 118     0     0:          *
>120     0     0:          *
122902592 residues in  1611 sequences
Statistics:  Expectation_n fit: rho(ln(x))= 7.6751+/-0.00204; mu= 6.7759+/- 0.231
 mean_var=233.8700+/-93.821, 0's: 0 Z-trim: 0  B-trim: 0 in 0/53
 Lambda= 0.083866
 Kolmogorov-Smirnov  statistic: 0.0247 (N=27) at  38
Algorithm: FASTA (3.5 Sept 2006) [optimized]
Parameters: +5/-4 matrix (5:-4) ktup: 6
 join: 52, opt: 37, open/ext: -12/-4, width:  16
 Scan time: 10.680
Enter filename for results []:  How many scores would you like to see? [20]

The best scores are:                                      opt bits E(1611)
gi|10727961|gb|AE003541.2|AE003541 Drosophila  (265536) [r]  171 36.0       1
gi|10728546|gb|AE003447.2|AE003447 Drosophila  (304085) [f]  171 36.0       1
gi|7290382|gb|AE003426.1|AE003426 Drosophila m (300193) [f]  159 34.5     2.8
gi|7290880|gb|AE003443.1|AE003443 Drosophila m (302357) [f]  157 34.3     3.3
```

```
gi|10727731|gb|AE003838.2|AE003838 Drosophila  (263411) [r]  149 33.3     6.4
gi|7291133|gb|AE003450.1|AE003450 Drosophila m (300732) [f]  148 33.2     6.9
gi|7300931|gb|AE003741.1|AE003741 Drosophila m (233313) [r]  151 33.2     7.1
gi|10726402|gb|AE003682.2|AE003682 Drosophila  (224400) [f]  147 33.1     7.5
gi|10728339|gb|AE003512.2|AE003512 Drosophila  (301457) [f]  147 33.1     7.5
gi|10728273|gb|AE003500.2|AE003500 Drosophila  (327446) [f]  145 32.8     8.9
gi|10726452|gb|AE003691.2|AE003691 Drosophila  (226773) [f]  145 32.8     8.9
gi|10727164|gb|AE003603.2|AE003603 Drosophila  (294914) [r]  144 32.6      10
gi|7290252|gb|AE003423.1|AE003423 Drosophila m (291976) [r]  144 32.6      10
gi|10727489|gb|AE003803.2|AE003803 Drosophila  (282567) [r]  143 32.6      10
gi|10727489|gb|AE003803.2|AE003803 Drosophila  (282567) [r]  143 32.5      11
gi|10727339|gb|AE003577.2|AE003577 Drosophila  (267662) [f]  142 32.3      13
gi|7292734|gb|AE003488.1|AE003488 Drosophila m (302797) [f]  140 32.2      13
gi|7298684|gb|AE003667.1|AE003667 Drosophila m (263704) [r]  139 31.9      17
gi|10727995|gb|AE003546.2|AE003546 Drosophila  (281602) [f]  137 31.9      17
gi|10728551|gb|AE003448.2|AE003448 Drosophila  (310364) [f]  137 31.9      18
 More scores? [0]
 Display alignments also? (y/n) [n]


560 residues in 1 query   sequences
122655632 residues in 1170 library sequences
 Scomplib [35.04]
 start: Wed Dec 10 19:45:41 2008 done: Wed Dec 10 19:46:04 2008
 Total Scan time: 10.680 Total Display time:  0.000

Function used was FASTA [version 35.04 Oct. 7, 2008]
```

3.7.3. Further Information

Further information about the usage of fasta can be obtained from /opt/bio/fasta/fasta3x.doc present on the frontend of your installation.

More information is also available at the FASTA home page[23].

For support, you are encouraged to join the FASTA mailing list at
http://list.mail.virginia.edu/mailman/listinfo/fasta_list

3.8. MrBayes

3.8.1. About

MrBayes is a program used for bayesian inference of phylogeny. MrBayes is cowritten by John Huelsenbeck and Fredrik Ronquist.

The version of MrBayes included with this version of Rocks is MPI enabled, and can be used in either parallel or serial modes of execution.

3.8.2. Usage

MrBayes uses the NEXUS file format for input. To use MrBayes in interactive mode, just type mb at the command line

```
[nostromo@xxx mrbayes]$ mb
MrBayes v3.1.2

                      (Bayesian Analysis of Phylogeny)

                            (Parallel version)
                         (1 processors available)

                                     by

                    Fredrik Ronquist and John P. Huelsenbeck

                          School of Computational Science
                              Florida State University
                               ronquist@csit.fsu.edu

                       Section of Ecology, Behavior and Evolution
                             Division of Biological Sciences
                          University of California, San Diego
                                johnh@biomail.ucsd.edu

                  Distributed under the GNU General Public License

                   Type "help" or "help <command>" for information
                         on the commands that are available.


MrBayes >
```

To use MrBayes in the parallel version, you'll need to use it in non-interactive mode. It can be invoked as shown.

```
[nostromo@xxx ~]$ /opt/openmpi/bin/mpirun -np 4 /opt/bio/mrbayes/mb /opt/bio/mrbayes/primates.nex
[nostromo@xxx ~]$ cat log.txt
                                  MrBayes v3.1.2

                      (Bayesian Analysis of Phylogeny)

                            (Parallel version)
                         (4 processors available)

                                     by

                    John P. Huelsenbeck and Fredrik Ronquist

                       Section of Ecology, Behavior and Evolution
                             Division of Biological Sciences
                          University of California, San Diego
                                johnh@biomail.ucsd.edu

                          School of Computational Science
                              Florida State University
                               ronquist@csit.fsu.edu
```

```
                Distributed under the GNU General Public License

                 Type "help" or "help <command>" for information
                       on the commands that are available.



   Executing file "/opt/bio/mrbayes/primates.nex"
   UNIX line termination
   Longest line length = 915
   Parsing file
   Expecting NEXUS formatted file
   Reading data block
      Allocated matrix
      Matrix has 12 taxa and 898 characters
      Data is Dna
      Data matrix is not interleaved
      Gaps coded as -
      Setting default partition (does not divide up characters).
      Taxon  1 -> Tarsius_syrichta
      Taxon  2 -> Lemur_catta
      Taxon  3 -> Homo_sapiens
      Taxon  4 -> Pan
      Taxon  5 -> Gorilla
      Taxon  6 -> Pongo
      Taxon  7 -> Hylobates
      Taxon  8 -> Macaca_fuscata
      Taxon  9 -> M_mulatta
      Taxon 10 -> M_fascicularis
      Taxon 11 -> M_sylvanus
      Taxon 12 -> Saimiri_sciureus
      Setting output file names to "/opt/bio/mrbayes/primates.nex.run<i>.<p/t>"
      Successfully read matrix
   Exiting data block
   Reached end of file

   Tasks completed, exiting program because mode is noninteractive
   To return control to the command line after completion of file processing,
   set mode to interactive with 'mb -i <filename>' (i is for interactive)
   or use 'set mode=interactive'

[nostromo@xxx ~]$
```

3.8.3. Further Information

A wealth of information about MrBayes is available at the MrBayes Home Page[24] and at the MrBayes Wiki[25]

3.9. Phylip

3.9.1. About

Phylip - Phylogeny Inference Package - is a package of programs for inferring phylogenies or evolutionary trees. The version distributed with Rocks is v3.68.

3.9.2. Further Information

Furtherinformation about Phylip is available at the Phylip home page[26].

3.10. T_Coffee

3.10.1. About

T_Coffee is a multiple sequence alignment package. The version included with this distribution of Rocks is v8.14

3.10.2. Usage

T-coffee is used for standard alignments and alignment combinations. It is installed at /opt/bio/tcoffee/ on the Rocks distribution. To use T-Coffee, just type t_coffee at the command line for a list of all possible parameters that can be used. T-coffee recognizes formats such as fasta, clustalw, blast, etc. Example input files are available at /opt/bio/tcoffee/example/

A simple sequence alignment example is shown below about. It is run against a sample fasta file present in the example directory. Parts of the output are deleted for the sake of brevity. Where missing, output is substituted by ellipses (.....)

```
[nostromo@xxx ~]$ t_coffee /opt/bio/tcoffee/example/sample_aln2.fasta

PROGRAM: T-COFFEE (Version_8.14)
-full_log       S       [0]
-run_name       S       [0]
-mem_mode       S       [0]     mem
-extend         D       [1]     1
-extend_mode    S       [0]     very_fast_triplet
-max_n_pair     D       [0]     10
-seq_name_for_quadruplet        S       [0]     all
-compact        S       [0]     default
-clean          S       [0]     no
-do_self        FL      [0]     0
-do_normalise   D       [0]     1000
-template_file  S       [0]
-template_mode  S       [0]
-remove_template_file   D       [0]     0
-profile_template_file  S       [0]
-in             S       [0]
-seq            S       [1]     /opt/bio/tcoffee/example/sample_aln2.fasta
-aln            S       [0]
```

```
-method_limits  S       [0]
-method         S       [0]
-lib            S       [0]
-profile        S       [0]
-profile1       S       [0]
-profile2       S       [0]
-pdb            S       [0]
-relax_lib      D       [0]     1
-filter_lib     D       [0]     0
-shrink_lib     D       [0]     0
-out_lib        W_F     [0]     no
-out_lib_mode   S       [0]     primary
-lib_only       D       [0]     0
-outseqweight   W_F     [0]     no
-dpa            FL      [0]     0
-seq_source     S       [0]     ANY
-cosmetic_penalty       D       [0]     0
-gapopen        D       [0]     0
-gapext         D       [0]     0
-fgapopen       D       [0]     0
-fgapext        D       [0]     0
-nomatch        D       [0]     0
-newtree        W_F     [0]     default
-tree           W_F     [0]     NO
-usetree        R_F     [0]
-tree_mode      S       [0]     nj
-distance_matrix_mode   S       [0]     ktup
-distance_matrix_sim_mode       S       [0]     idmat_sim1
-quicktree      FL      [0]     0
-outfile        W_F     [0]     default
-maximise       FL      [1]     1
-output         S       [0]     aln     html
-infile         R_F     [0]
-matrix         S       [0]     default
-tg_mode        D       [0]     1
-profile_mode   S       [0]     cw_profile_profile
-profile_comparison     S       [0]     profile
-dp_mode        S       [0]     linked_pair_wise
-ktuple         D       [0]     1
-ndiag          D       [0]     0
-diag_threshold D       [0]     0
-diag_mode      D       [0]     0
-sim_matrix     S       [0]     vasiliky
-transform      S       [0]
-outorder       S       [0]     input
-inorder        S       [0]     aligned
-seqnos         S       [0]     off
-case           S       [0]     keep
-cpu            D       [0]     0
-maxnseq        D       [0]     1000
-maxlen         D       [0]     -1
-weight         S       [0]     default
-seq_weight     S       [0]     t_coffee
-align          FL      [1]     1
-mocca          FL      [0]     0
-domain         FL      [0]     0
-start          D       [0]     0
```

```
-len            D       [0]     0
-scale          D       [0]     0
-mocca_interactive      FL      [0]     0
-method_evaluate_mode   S       [0]     default
-evaluate_mode  S       [0]     t_coffee_fast
-get_type       FL      [0]     0
-clean_aln      D       [0]     0
-clean_threshold        D       [1]     1
-clean_iteration        D       [1]     1
-clean_evaluate_mode    S       [0]     t_coffee_fast
-extend_matrix  FL      [0]     0
-prot_min_sim   D       [0]     0
-prot_max_sim   D       [90]    90
-prot_min_cov   D       [0]     0
-pdb_min_sim    D       [35]    35
-pdb_max_sim    D       [100]   100
-pdb_min_cov    D       [50]    50
-pdb_blast_server       W_F     [0]     EBI
-blast          W_F     [0]
-blast_server   W_F     [0]     EBI
-pdb_db         W_F     [0]     pdb
-protein_db     W_F     [0]     uniprot
-method_log     W_F     [0]     no
-struc_to_use   S       [0]
-cache          W_F     [0]     use
-align_pdb_param_file   W_F     [0]     no
-align_pdb_hasch_mode   W_F     [0]     hasch_ca_trace_bubble
-external_aligner       S       [0]     NO
-msa_mode       S       [0]     tree
-one2all        S       [0]
-subset2all     S       [0]
-lalign_n_top   D       [0]     10
-iterate        D       [0]     0
-trim           D       [0]     0
-split          D       [0]     0
-trimfile       S       [0]     default
-split          D       [0]     0
-split_nseq_thres       D       [0]     0
-split_score_thres      D       [0]     0
-check_pdb_status       D       [0]     0
-clean_seq_name D       [0]     0
-seq_to_keep    S       [0]
-dpa_master_aln S       [0]
-dpa_maxnseq    D       [0]     0
-dpa_min_score1 D       [0]
-dpa_min_score2 D       [0]
-dpa_keep_tmpfile       FL      [0]     0
-dpa_debug      D       [0]     0
-multi_core     S       [0]     templates_jobs_relax_msa
-n_core         D       [0]     0
-lib_list       S       [0]
-prune_lib_mode S       [0]     5
-tip            S       [0]     one
-rna_lib        S       [0]
-no_warning     D       [0]     0
-run_local_script       D       [0]     0
-plugins        S       [0]     default
```

```
-proxy          S       [0]     unset
-email          S       [0]
-clean_overaln  D       [0]     0
-overaln_param  S       [0]
-overaln_mode   S       [0]
-overaln_model  S       [0]
-overaln_threshold      D       [0]     0
-overaln_target D       [0]     0
-overaln_P1     D       [0]     0
-overaln_P2     D       [0]     0
-overaln_P3     D       [0]     0
-overaln_P4     D       [0]     0
-exon_boundaries        S       [0]

INPUT FILES
        Input File (S) /opt/bio/tcoffee/example/sample_aln2.fasta  Format clustal_aln
        Input File (M) proba_pair

INPUT SEQUENCES: 6 SEQUENCES  [PROTEIN]
  Input File /opt/bio/tcoffee/example/sample_aln2.fasta Seq 1cms   Length  175 type PROTEIN Struc
  Input File /opt/bio/tcoffee/example/sample_aln2.fasta Seq 4pep   Length  174 type PROTEIN Struc
  Input File /opt/bio/tcoffee/example/sample_aln2.fasta Seq 4ape   Length  178 type PROTEIN Struc
  Input File /opt/bio/tcoffee/example/sample_aln2.fasta Seq 3app   Length  174 type PROTEIN Struc
  Input File /opt/bio/tcoffee/example/sample_aln2.fasta Seq 2apr   Length  178 type PROTEIN Struc
  Input File /opt/bio/tcoffee/example/sample_aln2.fasta Seq 1cms_1 Length  148 type PROTEIN Struc

COMPUTE PAIRWISE SIMILARITY [dp_mode: ] [distance_matrix_mode: ktup][Similarity Measure: idmat_si

        Seq: 1cms
        Seq: 1cms_1
        Seq: 2apr
        Seq: 3app
        Seq: 4ape
        Seq: 4pep

READ/MAKE LIBRARIES:[2]

        proba_pair [method]

        Multi Core Mode: 2 processors [subset]

                [Submit   Job][TOT=    8][100 %][ELAPSED TIME:    0 sec.]
MANUAL PENALTIES: gapopen=0 gapext=0

        Library Total Size: [6175]

Library Relaxation: Multi_proc [2]
                [Submit   Job][TOT= 3087][100 %][ELAPSED TIME:    0 sec.]
Total Relaxation: [6175]--->[5092] Entries

        #### File Type=     WEIGHT Format=  tc_weight Name= no | NOT PRODUCED [WARNING:T-COFFEE:V


WEIGHTED MODE:t_coffee

          1cms 1.00
        1cms_1 1.10
```

```
          2apr 1.00
          3app 0.96
          4ape 0.95
          4pep 0.99


MAKE GUIDE TREE
        [MODE=nj][DONE]

PROGRESSIVE_ALIGNMENT [Tree Based]

        Group    8: [Group    5 (   1 seq)] with [Group    4 (   1 seq)]-->[Score=  83][Len=  179
        Group    7: [Group    6 (   1 seq)] with [Group    1 (   1 seq)]-->[Score=  92][Len=  176
        Group    9: [Group    8 (   2 seq)] with [Group    3 (   1 seq)]-->[Score=  74][Len=  186
        Group   10: [Group    9 (   3 seq)] with [Group    7 (   2 seq)]-->[Score=  77][Len=  186
        Group   11: [Group    2 (   1 seq)] with [Group   10 (   5 seq)]-->[Score=  24][Len=  209

CLUSTAL FORMAT for T-COFFEE Version_8.14 [http://www.tcoffee.org] [MODE:  ], CPU=0.15 sec, SCORE=

1cms            GE---VASVPLTNY------LDSQYFGKIYLGTPPQEFTVLFDTGSSDFWVPSIYCKSNA
4pep            -----IGDEPLENY------LDTEYFGTIGIGTPAQDFTVIFDTGSSNLWVPSVYCSSLA
4ape            S-TGSATTTPID-S------LDDAYITPVQIGTPAQTLNLDFDTGSSDLWVFSSETTASE
3app            AASGVATNTPTA--------NDEEYITPVTIGG--TTLNLNFDTGSADLWVFSTELPASQ
2apr            AG---VGTVPMTDY-----GNDIEYYGQVTIGTPGKKFNLDFDTGSSDLWIASTLCTN-C
1cms_1          Y-TGSLHWVPVTVQQYWQFTVDSVTISGVVVACEG-GCQAILDTGTSKLVGPSSD-----
                         *           *      : :.         :***::.:   *

1cms            CKNHQRFDPRKSSTFQ-NLGKPLSIHYGTGS-MQGILGYDTVTVSNIVDIQQTVGLSTQE
4pep            CSDHNQFNPDDSSTFE-ATSQELSITYGTGS-MTGILGYDTVQVGGISDTNQIFGLSETE
4ape            VDGQTIYTPSKSTTAKLLSGATWSISYGDGSSSSGDVYTDTVSVGGLTVTGQAVESAKKV
3app            QSGHSVYNPSATG-KE-LSGYTWSISYGDGSSASGNVFTDSVTVGGVTAHGQAVQAAQQI
2apr            GSGQTKYDPNQSSTYQ-ADGRTWSISYGDGSSASGILAKDNVNLGGLLIKGQTIELAKRE
1cms_1          ----------------------------------------------ILNIQQAIGATQNQ
                                                             :    * .  :

1cms            PGDVFTYAEFD--------GILGMAYPSLASEY-------SIPVFDNM-MNRHLVA----
4pep            PGSFLYYAPFD--------GILGLAYPSISASG-------ATPVFDNL-WDQGLVS----
4ape            SSSFTEDSTID--------GLLGLAFSTLNTVSPTQ----QKTFFDNA---KASLD----
3app            SAQFQQDTNND--------GLLGLAFSSINTVQPQS----QTTFFDTV---KSSLA----
2apr            AASFAS-GPND--------GLLGLGFDTITTVRG------VKTPMDNL-ISQGLIS----
1cms_1          YGEFDIDCDNLSYMPTVVFEINGKMYPLTPSAYTSQDQGFCTSGFQSENHSQKWILGDVF
                 ...               : *  :    :           . ::.    :  :

1cms            -QDLFSVYMDRN-G-QESMLTLGAIDPSY
4pep            -QDLFSVYLSSN-DDSGSVVLLGGIDSSY
4ape            -SPVFTADLGY---HAPGTYNFGFIDTTA
3app            -QPLFAVALKH---QQPGVYDFGFIDSSK
2apr            -RPIFGVYLGKAKNGGGGEYIFGGYDSTK
1cms_1          IREYYSVFDR--------ANNLVGLAKAI
                    : .             :     :



OUTPUT RESULTS
        #### File Type= GUIDE_TREE Format=     newick Name= sample_aln2.dnd
```

```
        #### File Type=        MSA Format=        aln Name= sample_aln2.aln
        #### File Type=        MSA Format=       html Name= sample_aln2.html


# TIP :See The Full Documentation on www.tcoffee.org
# TIP  1:  Get the most accurate protein alignments with: t_coffee <yourseq> -special_mode accura
# TIP  4:  -special_mode=expresso to fetch your structures automatically

# Command Line: t_coffee /opt/bio/tcoffee/example/sample_aln2.fasta  [PROGRAM:T-COFFEE]
# T-COFFEE Memory Usage: Current= 11.819 Mb, Max= 13.181 Mb
# T-COFFEE CPU Usage: 160 millisec
# Results Produced with T-COFFEE (Version_8.14)
# T-COFFEE is available from http://www.tcoffee.org
```

3.10.3. Further Information

Further information about t_coffee is available at -

- The T-coffee home page[27]
- On your cluster head node at /opt/bio/tcoffee/doc/
- T-Coffee Documentation[28]

3.11. TIGR Assembler v2

3.11.1. About

The TIGR Assembler is a tool to assemble large shotgun sequencing projects. The version included with this distribution of Rocks is v2

3.11.2. Usage

TIGR is used for assembling large shotgun DNA sequences. It is installed at /opt/bio/tigr on the Rocks Distribution. To use TIGR, just type TIGR_Assembler at the command line for a list of all possible parameters that can be used.

3.11.3. Further Information

Further information is available at the JCVI TIGR Assembler page[29]

3.12. MPI-Blast

3.12.1. About

MPI-Blast is a program from LANL[30] which parallelizes the NCBI Blast algorithms using Message Passing Interface library. The version of MPI-Blast included with Rocks is v1.5.0-pio patched and compiled against NCBI Blast 2.2.19.

3.12.2. Usage

MPI-Blast is used in a similar manner to NCBI-Blast. MPI-Blast uses the same variables that are available for NCBI-Blast.

There are 3 steps to running MPI-Blast.

- Download a FASTA database to $BLASTDB. For this example we will download the ecoli nucleotide database.

```
[nostromo@xxx ~]$ cd $BLASTDB
[nostromo@xxx ~]$ wget ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/ecoli.nt.gz
--17:06:23--  ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/ecoli.nt.gz
           => 'ecoli.nt.gz'
Resolving ftp.ncbi.nlm.nih.gov... 165.112.7.10
Connecting to ftp.ncbi.nlm.nih.gov|165.112.7.10|:21... connected.
Logging in as anonymous ... Logged in!
==> SYST ... done.    ==> PWD ... done.
==> TYPE I ... done.  ==> CWD /blast/db/FASTA ... done.
==> PASV ... done.    ==> RETR ecoli.nt.gz ... done.
Length: 1,438,199 (1.4M) (unauthoritative)

100%[======================================================>] 1,438,199
610.14K/s

17:06:27 (607.91 KB/s) - 'ecoli.nt.gz' saved [1438199]
```

- Format the database using mpiformatdb as follows. A good rule is to format the database to atleast 4 processors, as follows.

```
[nostromo@xxx ~]$ gunzip ecoli.nt.gz
[nostromo@xxx ~]$ ls
ecoli.nt
[nostromo@xxx ~]$ mpiformatdb --nfrags=4 -i ecoli.nt -pF --quiet
Reading input file
Done, read 58882 lines
Reordering 400 sequence entries
Breaking ecoli.nt into 4 fragments
Executing: formatdb -p F -i /tmp/reorderncq8B1 -N 4 -n /home/nostromo/bio/ncbi/db/ecoli.nt -o T
Removed /tmp/reorderncq8B1
Created 4 fragments.
[nostromo@xxx ~]$ ls
ecoli.nt          ecoli.nt.000.nsq  ecoli.nt.001.nsq  ecoli.nt.002.nsq  ecoli.nt.003.nsq
ecoli.nt.000.nhr  ecoli.nt.001.nhr  ecoli.nt.002.nhr  ecoli.nt.003.nhr  ecoli.nt.mbf
ecoli.nt.000.nin  ecoli.nt.001.nin  ecoli.nt.002.nin  ecoli.nt.003.nin  ecoli.nt.nal
ecoli.nt.000.nnd  ecoli.nt.001.nnd  ecoli.nt.002.nnd  ecoli.nt.003.nnd  formatdb.log
ecoli.nt.000.nni  ecoli.nt.001.nni  ecoli.nt.002.nni  ecoli.nt.003.nni
ecoli.nt.000.nsd  ecoli.nt.001.nsd  ecoli.nt.002.nsd  ecoli.nt.003.nsd
```

```
ecoli.nt.000.nsi  ecoli.nt.001.nsi  ecoli.nt.002.nsi  ecoli.nt.003.nsi
```

- Now create a test sequence file and run mpiblast on the sequence against the formatted database.

```
[nostromo@xxx ~]$ cat > test.txt
>Test
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGC
TTCTGAACTGGTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAA
TATAGGCATAGCGCACAGACAGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACC
ATTACCACCACCATCACCATTACCACAGGTAACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAG
CCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGGTAACGAGGTAACAACCATGCGAGTGTTGAA
GTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCGATATTCTGGAAAGCAATGCC
AGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGGTGGCGATGATTG
AAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT

[nostromo@xxx mpiblast]$ /opt/openmpi/bin/mpirun -np 4 /opt/bio/mpiblast/bin/mpiblast -d ecoli.
```

After mpirun terminates, result.txt contains the result of your computation.

3.12.3. Running MPI Blast and SGE

This section gives a brief overview of running MPI Blast with SGE

- Create a simple SGE submission scripts called mpiblast_sge.sh with the following contents

```
#!/bin/bash

#$ -cwd
#$ -j y
#$ -S /bin/bash

export MPI_DIR=/opt/openmpi/
export BLASTDB=$HOME/bio/ncbi/db/
export BLASTMAT=/opt/bio/ncbi/data/

$MPI_DIR/bin/mpirun -np $NSLOTS \
 /opt/bio/mpiblast/bin/mpiblast \
 -d ecoli.nt -i $HOME/test.txt \
 -p blastn -o $HOME/result.txt
```

- Run

```
[nostromo@xxx ~]$ qsub -pe orte 4 mpiblast_sge.sh
Your job 11 ("mpiblast_sge.sh") has been submitted
```

- The results of your computation will be present in $HOME/result.txt

Please note that an MPI blast job requires atleast 3 processors to run. The argument for mpirun specifying the number of processors should be factor of the number of pieces the blast database was divided into. If you're running on a cluster with 2 processors, SGE, by default, will not schedule a job which requires more than 2 slots to run.

3.12.4. Further Information

Further information about using mpiblast can be found at the MPI-Blast home page[31].

For support, please join the mpiblast mailing list[32]

3.13. GROMACS

3.13.1. About

GROMACS - Groningen MAchine for Chemical Simulation - is a software suite meant for molecular dynamics simulation.

The version of GROMACS included with the distribution is version 4.0.5. It is available at http://www.gromacs.org under the GNU General Public Licence v2.0.

3.13.2. Usage

GROMACS is setup in /opt/bio/gromacs directory. The version included in this distribution is compiled with mpi support. OpenMPI v1.3.3 is used as the MPI library.

To get more help on using GROMACS, please refer to the following resources:

- GROMACS Home Page[33]
- GROMACS Documentation[34]
- GROMACS Online Reference Manual[35]
- GROMACS FAQ[36]
- Tutorials available on your machines at /opt/bio/gromacs/share/tutor

3.14. Bioperl

3.14.1. About

Bioperl is a set of perl modules for Bio-informatics computation.

3.14.2. Usage

Bioperl modules can be used to supplement already existing applications such as t_coffee, clustalw, and blast. For information on how to use the library, please refer to the API Docs[37].

3.14.3. Further Information

Further information about bioperl is available at the Bioperl home page[38]

3.15. Biopython

3.15.1. About

Biopython is a set of python modules for Bio-informatics computation.

3.15.2. Usage

Biopython modules can be used to supplement already existing applications such as blast. For information on how to use the library, please refer to the biopython documentation[39].

3.15.3. Further Information

Further information about biopython is available at the Biopython home page[40]

Notes

1. http://hmmer.janelia.org/
2. http://www.ncbi.nlm.nih.gov/BLAST/
3. http://mpiblast.lanl.gov/
4. www.biopython.org
5. http://www.ebi.ac.uk/clustalw/
6. http://mrbayes.csit.fsu.edu/
7. http://www.tcoffee.org/Projects_home_page/t_coffee_home_page.html
8. http://emboss.sourceforge.net/
9. http://evolution.genetics.washington.edu/phylip.html
10. http://fasta.bioch.virginia.edu/
11. http://hmmer.janelia.org/#download
12. ftp://selab.janelia.org/pub/software/hmmer/CURRENT/Userguide.pdf
13. ftp://ftp.ncbi.nlm.nih.gov/blast/db/
14. ftp://ftp.ncbi.nlm.nih.gov/blast/db/
15. ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/
16. ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/
17. http://www.ncbi.nlm.nih.gov/BLAST/
18. /blast/docs/

19. http://www.ncbi.nlm.nih.gov/blast/BLAST_guide.pdf
20. http://emboss.sourceforge.net/
21. http://emboss.sourceforge.net/support/
22. http://www.cbcb.umd.edu/software/glimmer/glim302notes.pdf
23. http://fasta.bioch.virginia.edu/
24. http://mrbayes.csit.fsu.edu/index.php
25. http://mrbayes.scs.fsu.edu/wiki/index.php/Manual
26. http://evolution.genetics.washington.edu/phylip.html
27. http://www.tcoffee.org/Projects_home_page/t_coffee_home_page.html
28. http://www.tcoffee.org/Documentation/t_coffee/t_coffee_technical.htm
29. http://www.jcvi.org/cms/publications/listing/abstract/article/tigr-assembler-a-new-tool-for-assembling-large-shotgun-sequencing-projects/
30. http://www.lanl.gov/
31. http://mpiblast.lanl.gov/
32. http://mpiblast.lanl.gov/Support.Lists.html
33. http://www.gromacs.org/
34. http://www.gromacs.org/gromacs/documentation/documentation.html
35. /gromacs/online.html
36. /gromacs/gmxfaq.html
37. http://doc.bioperl.org/
38. http://www.bioperl.org/
39. http://biopython.org/DIST/docs/tutorial/Tutorial.html
40. http://www.biopython.org/

Appendix A. Rocks® Copyright

Appendix B. Third Party Copyrights and Licenses

This section enumerates the licenses from all the third party software components of this Roll. A "best effort" attempt has been made to insure the complete and current licenses are listed. In the case of errors or ommisions please contact the maintainer of this Roll. For more information on the licenses of any components please consult with the original author(s) or see the Rocks CVS repository[1].

B.1. Biopython

```
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```

B.2. Clustal W

```
Date:29 November 2007

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B.7. HMMER

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