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.
Table 1-1. Summary

<table>
<thead>
<tr>
<th>Name</th>
<th>bio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Version</td>
<td>6.2</td>
</tr>
<tr>
<td>Maintained By</td>
<td>Rocks Group</td>
</tr>
<tr>
<td>Architecture</td>
<td>i386, x86_64</td>
</tr>
<tr>
<td>Compatible with Rocks®</td>
<td>6.2</td>
</tr>
</tbody>
</table>

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

Table 1-2. Compatibility

<table>
<thead>
<tr>
<th>Requires</th>
<th>Conflicts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td></td>
</tr>
<tr>
<td>HPC</td>
<td></td>
</tr>
<tr>
<td>Kernel</td>
<td></td>
</tr>
<tr>
<td>OS</td>
<td></td>
</tr>
<tr>
<td>Web Server</td>
<td></td>
</tr>
</tbody>
</table>
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.

![Welcome to Rocks](image)

### Selected Rolls

<table>
<thead>
<tr>
<th>Roll Name</th>
<th>Version</th>
<th>Arch</th>
</tr>
</thead>
<tbody>
<tr>
<td>kernel</td>
<td>4.2</td>
<td>x86_64</td>
</tr>
</tbody>
</table>

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
```
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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/
- 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
- Emboss - From European Molecular Biology Institute - http://emboss.sourceforge.net/
- Phylib - From the Dept. of Biology at the University of Washington - http://evolution.genetics.washington.edu/phylib.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.cbc.bcmd.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\textsuperscript{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\textsuperscript{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 matrices 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/.
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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 ~]$ gunzip -c nt.08.tar.gz | ( cd $BLASTDB/ && tar -xf - )

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


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 $BLASTDB
[nostromo@xxx ~]$ 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
TTCTGAACTGGTTAATTCCCGTGAAATTATTATTATTATTATTATTATTATTATTATTATTATTAT
TATAGCCGACACCGAAATAAAATATACAGTGAAAGCTGATGAAATGAAAATAGAAAATAGAAAATAG
ATTACACACACCGAGCGGTCGCTGATGAAATGAAAATAGAAAATAGAAAATAG
CCCGACACACACCGAGCGGTCGCTGATGAAATGAAAATAGAAAATAGAAAATAG

- 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]

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Query = Test

(Database: drosoph.nt

1170 sequences; 122,655,632 total letters

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

Score E

Sequences producing significant alignments:

Score (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 | 7297554|gb|AE003484.1|AE003484 Drosophila melanogaster genomi... | 34 | 3.4 |
| gi | 10727872|gb|AE003525.2|AE003525 Drosophila melanogaster genomi... | 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 -

```bash
#!/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
```

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- Run

```bash
[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

- BLAST Help page on your cluster BLAST Help Page

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

```bash
[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.

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. 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
  
  ```bash
  [nostromo@xxx ~]$ cat > test.txt
  >Test
  AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGC
  TTCTGAACACTGTACCTGCTGGTGAGTAATTTTTTATTACGCTGTCACCTAAATACTTTAAAACCA
  TATAGGGCATAGCGACAGACAGATATATAATTACAGAGTACACACACATCAGAAAACGCATAGCACCACC
  ATTTACCACTACACATACCACAGGTACAGGTGGCCTACACAGGAAACACAGA
  CCGACACCTGACAGTGCGGGCTTTTTTTTCGACCAAAGGTAACGAGGTAACAACCATGCGAGTGTTGAA
  GTTCGGGGTACAGTGGAGAATGAGAAGATCTGGTTGGCCGATATCTGGAAAGCAATGGC
  AGGCAGGGGAGTTGACCACCGCTCTCTGCCCACCACAAAATCAACACCTGCGAGGTGAT
  AAAAAACCAATAGCCGCCAGGATGCTTTACGATACCTGACGATGCGAAGAGA
  9
  ```

- 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/.

  ```bash
  [nostromo@xxx ~]$ fasta35
  # fasta35
  FASTA searches a protein or DNA sequence data bank
  version 35.04 Oct. 7, 2008
  Please cite:
  
  test sequence file name: test.txt
  library file name: drosoph.nt
  ktup? (1 to 6) [6]
  opt E()
  Query: test.txt
  1>>>Test - 560 nt
  Library: drosoph.nt
  ....... Done!
  122655632 residues in 1170 sequences
  ```

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

| gi|107727961|gb|AE003541.2|AE003541 Drosophila (265536) [r] | 171 36.0 1 |
| gi|107728546|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 |
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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.

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)

(Please version)

by

Fredrik Ronquist and John P. Huelsenbeck

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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

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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)

(Please version)

by

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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 Page24 and at the MrBayes Wiki25
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

Further information about Phylip is available at the Phylip home page³⁶.

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]
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- -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] use
- -cache W_F [0] use
- -align_pdb_param_file W_F [0] no
- -align_pdb_hash_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
Chapter 3. Using

```
-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 Struct Unchecked
Input File /opt/bio/tcoffee/example/sample_aln2.fasta Seq 4pep Length 174 type PROTEIN Struct Unchecked
Input File /opt/bio/tcoffee/example/sample_aln2.fasta Seq 4ape Length 178 type PROTEIN Struct Unchecked
Input File /opt/bio/tcoffee/example/sample_aln2.fasta Seq 3app Length 174 type PROTEIN Struct Unchecked
Input File /opt/bio/tcoffee/example/sample_aln2.fasta Seq 2apr Length 178 type PROTEIN Struct Unchecked
Input File /opt/bio/tcoffee/example/sample_aln2.fasta Seq 1cms_1 Length 148 type PROTEIN Struct Unchecked

COMPUTE PAIRWISE SIMILARITY [dp_mode: ] [distance_matrix_mode: ktup][Similarity Measure: idmat_sim1]
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:Version_8.14

WEIGHTED MODE:t_coffee

1cms 1.00
1cms_1 1.10
```
Chapter 3. Using

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= 176]
Group 10: [Group 9 (3 seq)] with [Group 7 (2 seq)] --> [Score= 77] [Len= 176]
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=72, Nseq=6, Len=209

1cms
GE---VASVPLTNY-------LDSQYFKIYLGTPPQEFTVLFDTGSSDFWVPSIYCKSNA
4pep
-----IGDEPLENY-------LDTEYFTGISGTPAQDFVIFTGSSNLWPSYCCSLSA
4ape
S-TGSATTPPID-S-------LDAYITPQIGTPAQTLNLDFDTGSSDLWVFSETTASE
3app
AASGVATNTPTA--------NDEEYITPVTGG------TTLNLNFDTGASLWVFSTELPAQ
2apr
AG----VTGTPMTDY------GNDIYYYQVTGTPKFKFLDFDTGSSDLWIASTLCTC
1cms_1
Y-TGSLHNPVVTQYQFTVDSVTISGVVVACEG--GCQAILDTGSKLVGSSD-----

1cms
CKNHQRFDPKSSFQ-NGKPLSIHYGTPS-MQGILGYDVTTVSNVDIQQTGVGLSTQE
4pep
CSDDHOFNDPDDSTFE-ATSQELSITYGTPS-MTGLILYDVTQVGIGIDTNQIFGLSETE
4ape
VDGQTIYTPSKSTTAKGLSASWGYSGYDSTVQSGLTVGQLAGCLKVE
3app
QSGHSSVNPSTAG-KE-LSGYTWSISYGDDSSASGNSVTFSDTVGVTAAQGAVQAQQI
2apr
GSQOTYKDPNQSTYQ-ADGRTWSISYGDDSSASILAKDNVLGGILLKGTIELAKRE
1cms_1
----------------------------------------------ILNIIQAIAGTQNGQ

1cms
PGDVFTYAEFD--------GILGMAYPSLASEY-------SIPVFDNM-MNRHLVA----
4pep
PGSLFYAPF--------GILGLAYPSISASG-------ATPVFDNL-NQGQVSNS----
4ape
SSFTESTID--------GGLLAFSTLNTVSPQG--QKTFDFNA---KASLDD----
3app
SAQFOQDTQTNND--------GGLLAFSINTVQOQQS--QTIPFDTV-------KSNS----
2apr
AASFSAP-GN--------GGLLGFDTTITVQRG------VKTPMDNL-ISQGLISL----
1cms_1
YGEFDPIDCDNLNSYMTVVFIEINGKYMPYLPSTAYTSQDGQFTCSTQFSQENHSGKWLGDVF

1cms
IREYSVFDV----------ANLNVLGAKAI

OUTPUT RESULTS

#### File Type= GUIDE_TREE Format= newick Name= sample_aln2.dnd
# 3.10.3. Further Information

Further information about t_coffee is available at -

- The T-coffee home page\(^\text{27}\)
- On your cluster head node at /opt/bio/tcoffee/doc/
- T-Coffee Documentation\(^\text{28}\)

# 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\(^\text{29}\)
3.12. MPI-Blast

3.12.1. About
MPI-Blast is a program from LANL 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
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.003.nsd
ecoli.nt.000.nsd ecoli.nt.001.nsd ecoli.nt.002.nsd ecoli.nt.003.nsd
```
Chapter 3. Using

3.12.3. Running MPI Blast and SGE

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

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

  [nostromo@xxx ~]$ cat > test.txt
  > AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGC
  > TTCTGAAACTGTATCACCTGCCGCTGAGTAATTAAAAATTTATGACTTTAGTCACATAAATATTAAACCAA
  > TATAGGCATAGCCACAGACAGATAAAAATTACAGAGTACAAACTTACCATGCAAACACGGTGACAGCAGGAGCA
  > TGACATCAATTCGGTGATATCAAGCTCGGTTCCTGACAGTGCGGGCTTTTTTTTCGACCAAAGGTAACGAGGA
  > GGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
  > AAAACATACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTT

  [nostromo@xxx mpiblast]$ /opt/openmpi/bin/mpirun -np 4 /opt/bio/mpiblast/bin/mpiblast -d ecoli.nt -i $HOME/test.txt -p blastn -o $HOME/result.txt

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

Please note that an MPI blast job requires at least 3 processors to run. The argument for mpirun specifying the number of processors should be a 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\textsuperscript{31}. For support, please join the mpiblast mailing list\textsuperscript{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\textsuperscript{33}
- GROMACS Documentation\textsuperscript{34}
- GROMACS Online Reference Manual\textsuperscript{35}
- GROMACS FAQ\textsuperscript{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\textsuperscript{37}. 
3.14.3. Further Information

Further information about bioperl is available at the Bioperl home page.

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.

3.15.3. Further Information

Further information about biopython is available at the Biopython home page.

Notes

1. http://hmmer.janelia.org/
4. www.biopython.org
5. http://www.ebi.ac.uk/clustalw/
11. http://hmmer.janelia.org/#download
18. /blast/docs/
Chapter 3. Using

35. /gromacs/online.html
36. /gromacs/gmxfaq.html
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Rocks(r)
www.rocksclusters.org
version 6.2 (SideWinder)

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Date: 29 November 2007

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B.3. EMBOSS

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BLAST - open source parallel BLAST

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