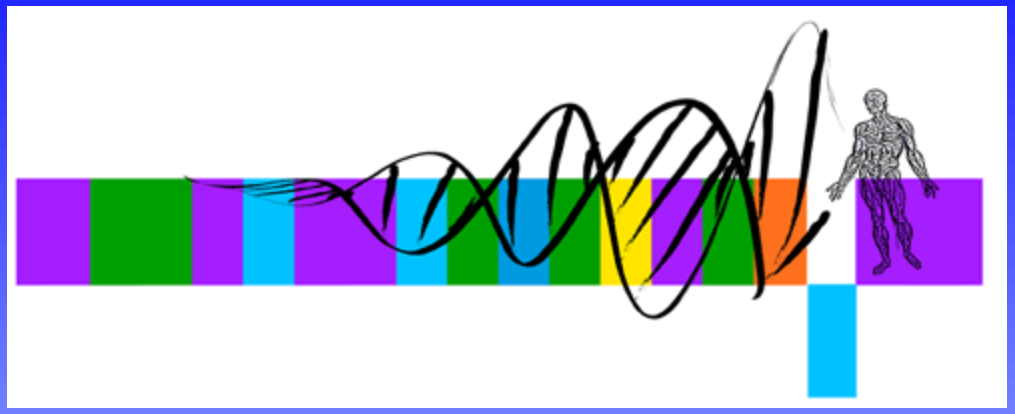

Unix, Perl and BioPerl

I: Introduction to Unix for Bioinformatics

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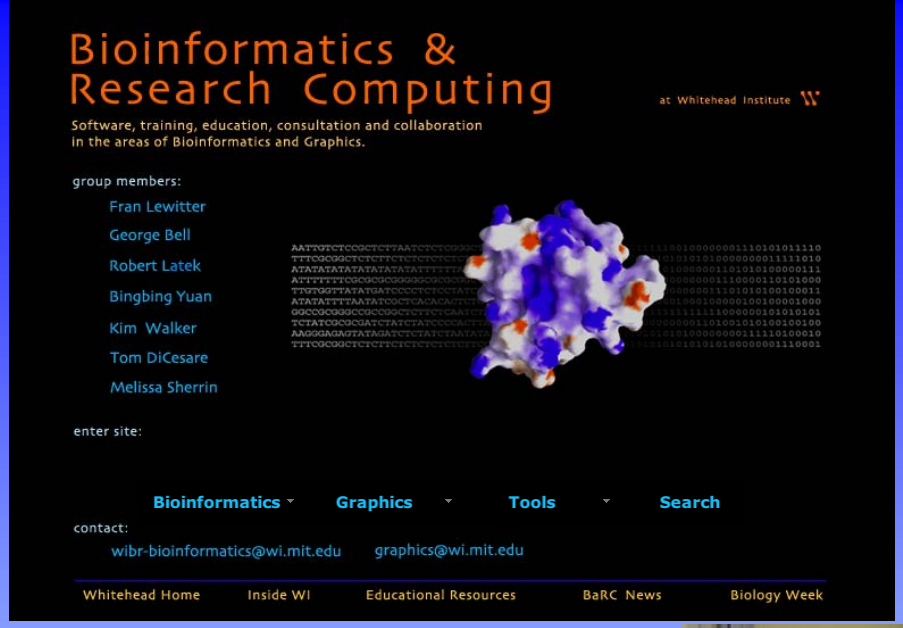
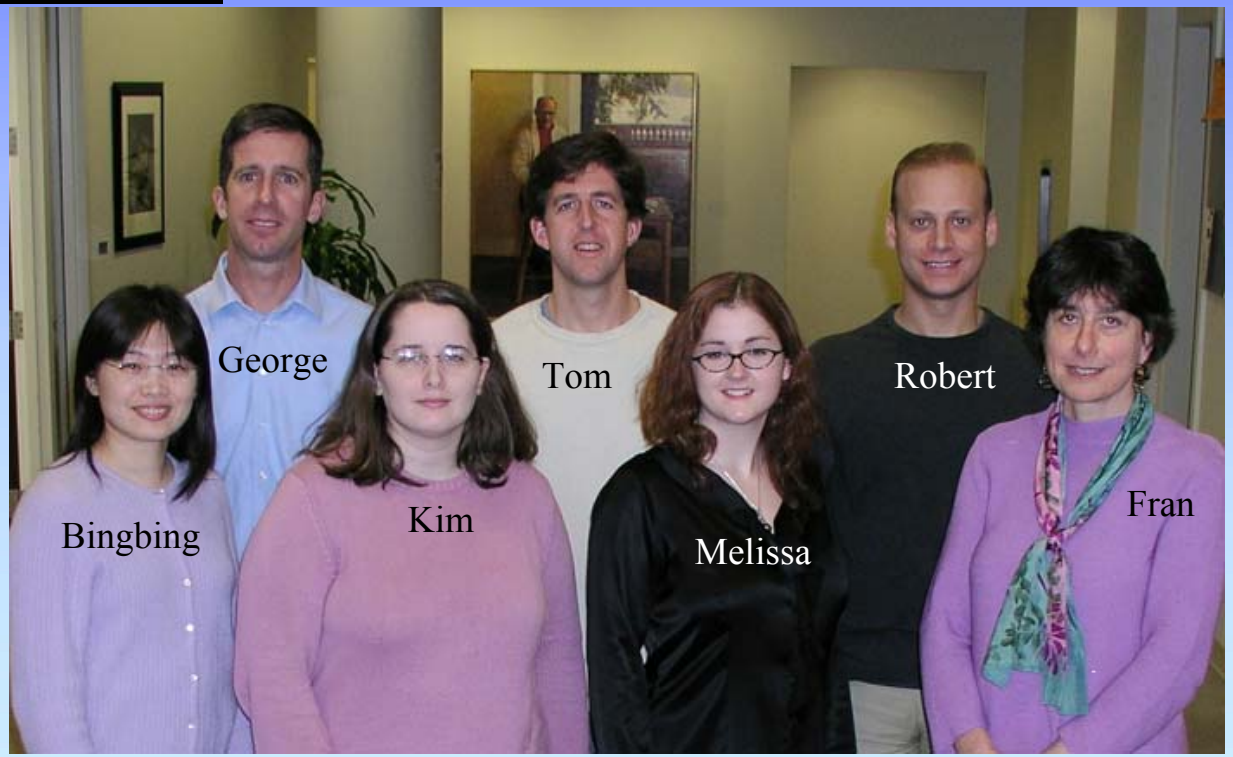
WIBR Bioinformatics and Research Computing



http://web.wi.mit.edu/bio



- Training
  - Train Whitehead scientists on the use of bioinformatics and graphics tools
- Education
  - Teach courses about theory behind bioinformatics tools and graphics concepts
- Consulting
  - Advise scientists on ways of analyzing data and designing graphics images
- Collaboration
  - Build new bioinformatics tools
  - Use bioinformatics tools to analyze research data
  - Publish papers in the area of bioinformatics with Whitehead scientists

Introduction to Unix for Bioinformatics

- Why Unix?
- The Unix operating system
- Files and directories
- Ten required commands
- Input/output and command pipelines
- Supplementary information
  - X windows
  - EMBOSS
  - Shell scripts

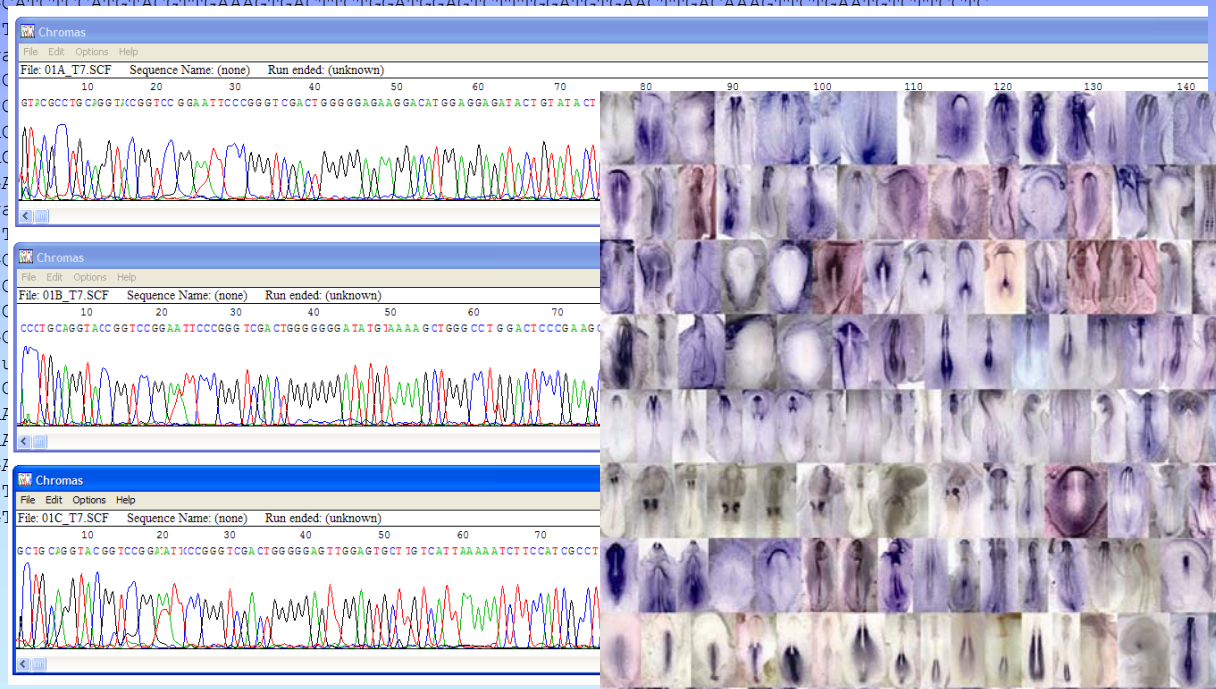
Objectives

- Get around on a Unix computer
- Run bioinformatics programs "from the command line"
- Design potential ways to streamline data manipulation and analysis with scripts

Why Unix (for me)?

- GEISHA, the *Gallus gallus* (chicken) EST and in situ hybridization (ISH) database

```
>A01_T3 | GEISHA | Gallus gallus | 496 nt | 77:572
ATCAAAGGCTTTACCGACAAACATCATTTGCACAATTAGTTGTTGGACAGGAGGGAGGACACCCGAGGACATGTAGGCTCGAGCCATAGTGTTGCCAAGGCTCTC
CCTGTTTGTTCCTTGGGTGAGCTGAGCCAACAGCTCTCCCTGCCCTCAGGAAGGCAGCAGTGGTGACAGGCACTCTATGGGGACTAACAGGAGGGGGTGGTTGTG
GTGACCTCGGAGCAGGCAGCATCTCACCCATCACTCACACTGCAGACAGCATCACTGTGAAGGCCTACAGATACTGCAGTGTGGGTCACAAAAGCATCCACTGGC
TGCTCCTCACCTCTTCTTCTTCCTCAGCATCTCCATGTACGTTGAAAGTGACTTCTGGATGGAGTCTTTGGATGTGAACTTGACAAAGTTCTGAATGTCTTCCTC
CCGGTGAGCAAGCATGTGGTCCAGCACT
>A02_T3 | GEISHA | Gallus ga
ACTTCTCGGTTTATTAAAAACGGATACC
GGGCCTCCTCTTCCTCTGCCGCGGCCCC
TCCACTAGCAAGGTGTCCAGGGGCAAAC
AGCGTCATTTTCACAGCCTTGAGATGAC
TGACTCAGCTTCATCAGAAACCTGACGA
>A03_T3 | GEISHA | Gallus ga
GCCGTCCCTCTTAATCATGGCCCCGTTT
AACACTCTAATTTTTTCAAAGTAAACGC
CCTCGCGGCGGACCGCCAGCTCGATCCC
ACCAGACTTGCCCTCCAATGGATCCTCC
CCCCGGGTCGGGAGTGGGTAATTTGCGC
>lcl|A05_T3 | GEISHA | Gallu
GCTGATTATGCCGTTGCAGAGCAGGTTC
AACACTTCCTTAGTATTTAAAAACAAAA
ACTGGGGTTGTTCACTGCTTACTTCTAA
ATTTACTTCAGTAACGTAGTTACAGAGA
CTCTGAATTAATTAAATATTTTAAAATT
CTGGGCTAATGCCCCAGCCTCCTCTAGT
```



Why Unix (in general)?

- Features: multiuser, multitasking, network-ready, robust
- Others use it – and you can benefit from them (open source projects, etc.)
- Good programming and I/O tools
- Scripts can be easily re-run
- Types: Linux, Solaris, Darwin, etc.
- Can be very inexpensive

Why Unix for Bioinformatics?

- Good for manipulating lots of data
- Many key tools written for Unix
- Don't need to re-invent the wheel
- Unix-only packages: EMBOSS, BioPerl
- Unix tools with other OSs: Mac (OS X) & PC (Cygwin)

Unix O.S.

- kernel
  - managing work, memory, data, permissions
- shell:
  - working environment and command interpreter
  - link between kernel and user
  - choices: tcsh, etc.
  - History, filename completion [tab], wildcard (*)
  - Shell scripts to combine commands
- filesystem
  - ordinary files, directories, special files, pipes

WIBR BaRC systems

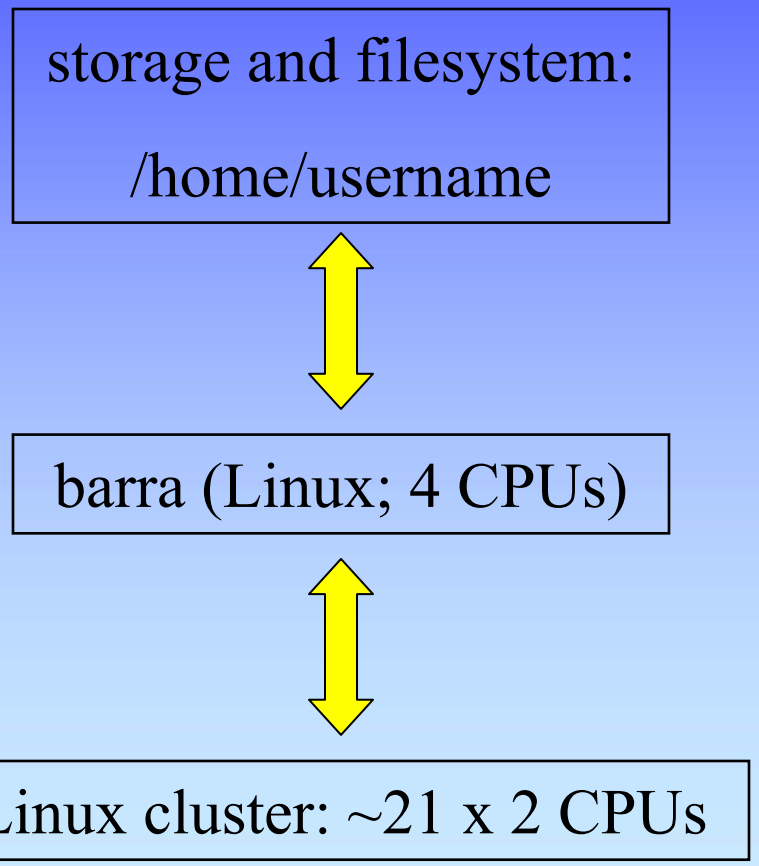
Training

hebrides

(Solaris; 2 CPUs)

with storage and filesystem /home/username

Research



Logging in

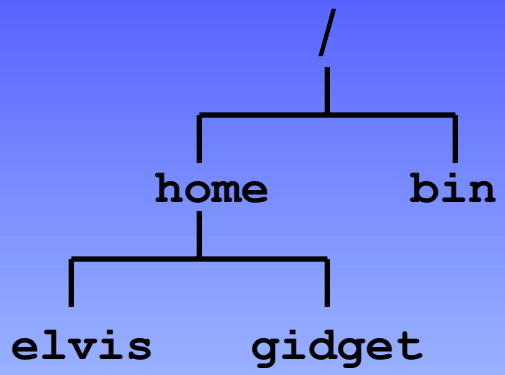
- ssh (secure shell; for encrypted data flow)
  `ssh -l user_name hebrides.wi.mit.edu`

- passwd: to change your passwd

- logging out
  `logout`

Intro to files and directories

- Arranged in a branching tree
- Root of tree at "/" directory
- User elvis lives at /home/elvis (on 'hebrides')
- No spaces allowed
- Full vs. relative pathnames
  - At his home, Elvis' home dir is "."
  - To get to /home/gidget, go up and back down: (../gidget relative to /home/elvis)
- Anywhere, your home directory is "~".



Intro to Unix commands

- Basic form is

command_name options argument(s)

examples:

```
mv old_data new_data
blastall -p blastn -i myFile.seq -e 0.05
   -d nt -T T -o myFile.out
```

- Use history (↑, ↓, !*num*) to re-use commands
- Cursor commands: ^A(beginning) and ^E (end)
- To get a blank screen: `clear`
- For info about a command: `man` *`command`*

Key commands p. 1

- **Where am I?**

```
elvis@hebrides[1]% pwd
/home/elvis
```

- **What's here?**

```
elvis@hebrides [2]% ls
A01.fa

elvis@hebrides [3]% ls -a
.       .cshrc          A01.fa
..      .twmrc

elvis@hebrides [4]% ls -l
-rw-r--r--  1 elvis musicians  1102 Jun 19 10:45 A01.fa
```

Key commands p. 2

- Change directories:
  `cd ../gidget`
  `/home/gidget`

- Make a new directory:
  `mkdir spleen`

- Remove a directory (needs to be empty first):
  `rmdir spleen`

File permissions

- Who should be reading, writing, and executing files?
- Three types of people: user (u), group (g), others (o)
- 9 choices (rwx or each type of person; default = 644)

0 = no permission	4 = read only
1 = execute only	5 = r + x
2 = write only	6 = r + w
3 = x + w	7 = r + w + x

- Setting permissions with chmod:

`chmod 744 myFile` or **`chmod u+x myFile`**

```
-rwxr--r--  1 elvis musicians  110 Jun 19 10:45 myFile
```

`chmod 600 myFile`

```
-rw-------  1 elvis musicians  110 Jun 19 10:45 myFile
```

Key commands p.3

- Copying a file:

`cp [OPTION]... SOURCE DEST`

`Ex: cp mySeq seqs/mySeq`

- Moving or renaming a file:

`mv [OPTION]... SOURCE DEST`

`Ex: mv mySeq seqs/mySeq`

- Looking at a file (one screenful) with 'more'

`Ex: more mySeq`

(Spacebar a screenful forward,
<enter> a line forward; ^B a screenful back; q to exit)

Key commands (summary)

ssh	**mkdir**	**cp**
pwd	**mvdir**	**mv**
ls	**chmod**	**more**
cd		

To get more info (syntax, options, etc.):
man ***command***

Input/output redirection

- Defaults: stdin = keyboard; stdout = screen
- To modify,

  `command < inputFile > outputFile`
- input examples

  `sort < my_gene_list`
- output examples

  `ls > file_name` (make new file)

  `ls >> file_name` (append to file)

  `ls foo >& file_name` (stderr **too**)

Pipes (command pipelines)

- In a pipeline of commands, the output of one command is used as input for the next

- Link commands with the "pipe" symbol: |
  ex1: **`ls *.fa | wc -l`**
  ex2: **`grep '^>' *.fa | sort`**

Managing jobs and processes

- Run a process in the foreground (fg):
  command
- Run a process in the background (bg):
  command &
- Change a process (fg to bg):
  1. suspend the process: **^Z**
  2. change to background: **bg**

Managing jobs and processes (cont.)

- See what's running (ps)

```
elvis@hebrides[1]% ps -u user_name
   PID TTY          TIME CMD
22541 pts/22    0:00 perl
22060 pts/22    0:00 tcsh
```

- Stop a process:

kill *PID*

ex: ***kill 22541***

Text editors

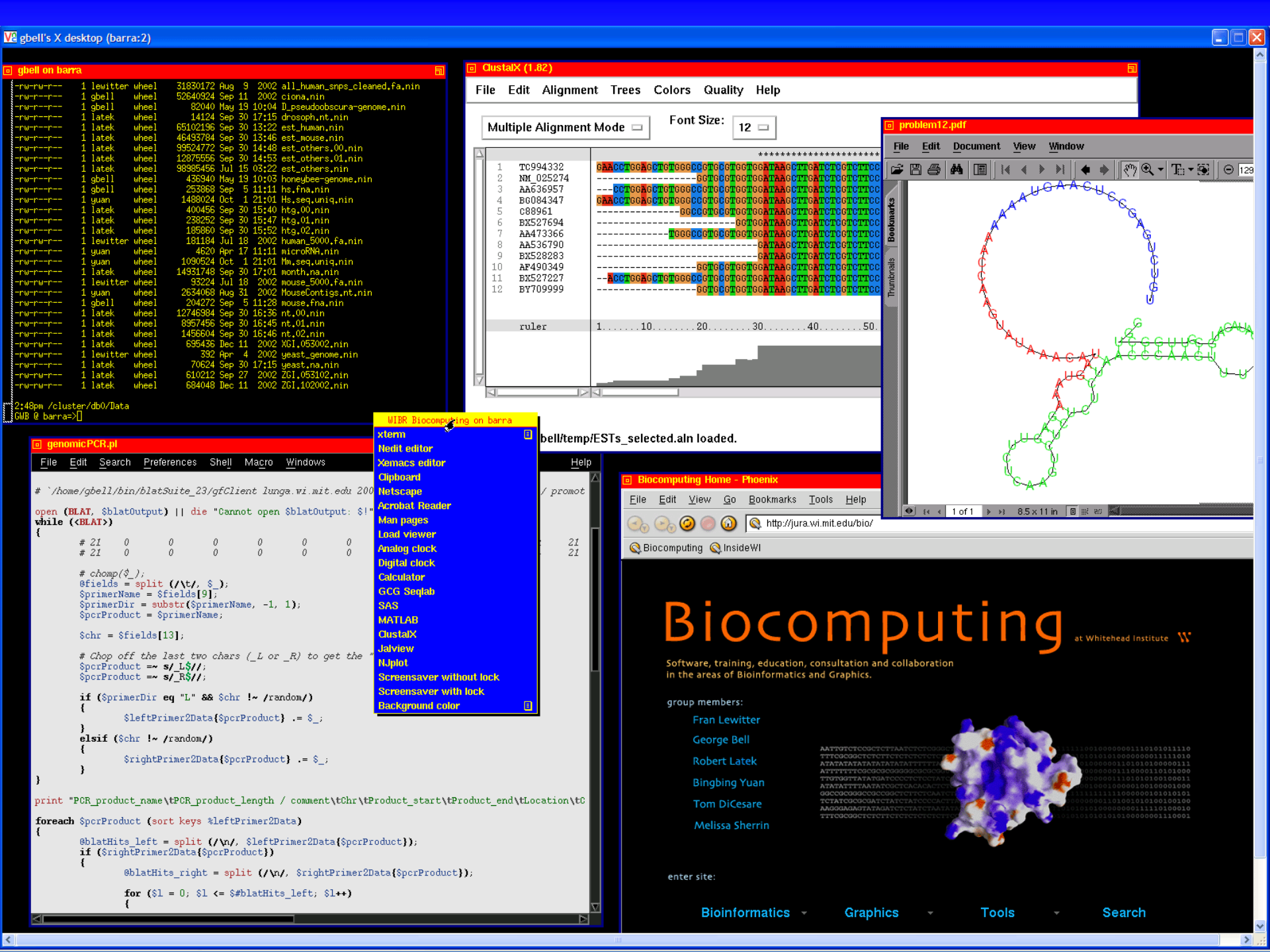
- emacs, vi (powerful but unfriendly at first); pico
- nedit, xemacs (easier; X windows only)
- desktop text editors (BBEdit; TextPad) + sftp

Supplementary information

X Windows

- method for running Unix graphical applications
- still allows for command-line operation
- see help pages for getting started
- some applications with extensive graphics:
  - EMBOSS
  - R
  - Matlab
  - ClustalX + TreeView

- Requires a fast network/internet connection


gbell's X desktop (barra:2)
gbell on barra
-rw-rw-r-- 1 lewitter wheel 31830172 Aug 9 2002 all_human_snps_cleaned.fa.nin
-rw-r--r-- 1 gbell wheel 52640924 Sep 11 2002 ciona.nin
-rw-r--r-- 1 gbell wheel 82040 May 19 10:04 D_pseudoobscura-genome.nin
-rw-r--r-- 1 latek wheel 14124 Sep 30 17:15 drosoph.nt.nin
-rw-r--r-- 1 latek wheel 65102196 Sep 30 13:22 est_human.nin
-rw-r--r-- 1 latek wheel 46493784 Sep 30 13:46 est_mouse.nin
-rw-r--r-- 1 latek wheel 99524772 Sep 30 14:48 est_others.00.nin
-rw-r--r-- 1 latek wheel 12875556 Sep 30 14:53 est_others.01.nin
-rw-r--r-- 1 latek wheel 98985456 Jul 15 03:22 est_others.nin
-rw-r--r-- 1 gbell wheel 436940 May 19 10:03 honeybee-genome.nin
-rw-r--r-- 1 gbell wheel 253868 Sep 5 11:11 hs.fna.nin
-rw-r--r-- 1 yuan wheel 1488024 Oct 1 21:01 Hs.seq.uniq.nin
-rw-r--r-- 1 latek wheel 400456 Sep 30 15:40 htg.00.nin
-rw-r--r-- 1 latek wheel 238252 Sep 30 15:47 htg.01.nin
-rw-r--r-- 1 latek wheel 185860 Sep 30 15:52 htg.02.nin
-rw-rw-r-- 1 lewitter wheel 181184 Jul 18 2002 human_5000.fa.nin
-rw-r--r-- 1 yuan wheel 4620 Apr 17 11:11 microRNA.nin
-rw-r--r-- 1 yuan wheel 1090524 Oct 1 21:01 Mm.seq.uniq.nin
-rw-r--r-- 1 latek wheel 14931748 Sep 30 17:01 month.na.nin
-rw-rw-r-- 1 lewitter wheel 93224 Jul 18 2002 mouse_5000.fa.nin
-rw-r--r-- 1 yuan wheel 2634068 Aug 31 2002 MouseContigs.nt.nin
-rw-r--r-- 1 gbell wheel 204272 Sep 5 11:28 mouse.fna.nin
-rw-r--r-- 1 latek wheel 12746984 Sep 30 16:36 nt.00.nin
-rw-r--r-- 1 latek wheel 8957456 Sep 30 16:45 nt.01.nin
-rw-r--r-- 1 latek wheel 1456604 Sep 30 16:46 nt.02.nin
-rw-rw-r-- 1 latek wheel 695436 Dec 11 2002 XGI.053002.nin
-rw-rw-r-- 1 lewitter wheel 392 Apr 4 2002 yeast_genome.nin
-rw-r--r-- 1 latek wheel 70624 Sep 30 17:15 yeast.na.nin
-rw-rw-r-- 1 latek wheel 610212 Sep 27 2002 ZGI.053102.nin
-rw-rw-r-- 1 latek wheel 684048 Dec 11 2002 ZGI.102002.nin
2:48pm /cluster/db0/Data
GWB @ barra>
ClustalX (1.82)
File Edit Alignment Trees Colors Quality Help
Multiple Alignment Mode
Font Size: 12
1 TC994332
2 NM_025274
3 AA636957
4 BG084347
5 C88961
6 BX527694
7 AA473366
8 AA536790
9 BX528283
10 AF490349
11 BX527227
12 BY709999
ruler 1.....10.....20.....30.....40.....50.
bell/temp/ESTs_selected.aln loaded.
problem12.pdf
File Edit Document View Window
Bookmarks
Thumbnails
1 of 1
8.5 x 11 in
WIBR Biocomputing on barra
xterm
Nedit editor
Xemacs editor
Clipboard
Netscape
Acrobat Reader
Man pages
Load viewer
Analog clock
Digital clock
Calculator
GCG Seqlab
SAS
MATLAB
ClustalX
Jalview
NJplot
Screensaver without lock
Screensaver with lock
Background color
genomicPCR.pl
File Edit Search Preferences Shell Macro Windows Help
`/home/gbell/bin/blatSuite_23/gfClient lunga.wi.mit.edu 200 / promot
open (BLAT, $blatOutput) || die "Cannot open $blatOutput: $!"
while ((<BLAT>))
{
# 21 0 0 0 0 0 0 21
# 21 0 0 0 0 0 0 21
# chomp($_);
@fields = split (/\t/, $_);
$primerName = $fields[9];
$primerDir = substr($primerName, -1, 1);
$pcrProduct = $primerName;
$chr = $fields[13];
Chop off the last two chars (_L or _R) to get the
$pcrProduct =~ s/_L$//;
$pcrProduct =~ s/_R$//;
if ($primerDir eq "L" && $chr !~ /random/)
{
$leftPrimer2Data{$pcrProduct} .= $_;
}
elsif ($chr !~ /random/)
{
$rightPrimer2Data{$pcrProduct} .= $_;
}
}
print "PCR_product_name\tPCR_product_length / comment\tChr\tProduct_start\tProduct_end\tLocation\tC
foreach $pcrProduct (sort keys %leftPrimer2Data)
{
@blatHits_left = split (/\n/, $leftPrimer2Data{$pcrProduct});
if ($rightPrimer2Data{$pcrProduct})
{
@blatHits_right = split (/\n/, $rightPrimer2Data{$pcrProduct});
for ($l = 0; $l <= $#blatHits_left; $l++)
{
Biocomputing Home - Phoenix
File Edit View Go Bookmarks Tools Help
http://jura.wi.mit.edu/bio/
Biocomputing InsideWI
Biocomputing
at Whitehead Institute
Software, training, education, consultation and collaboration
in the areas of Bioinformatics and Graphics.
group members:
Fran Lewitter
George Bell
Robert Latek
Bingbing Yuan
Tom DiCesare
Melissa Sherrin
enter site:
Bioinformatics
Graphics
Tools
Search

EMBOSS

- The European Molecular Biology Open Software Suite
- List of programs at http://www.hgmp.mrc.ac.uk/Software/EMBOSS/Apps/
- ex: Smith-Waterman local alignment (`water`)
- Programs have two formats: interactive and one-line
- Conducive to embedding in scripts for batch analysis
- Traditionally command-line but web interfaces are becoming available

EMBOSS examples

- needle: Needleman-Wunsch global alignment

```
needle seq1.fa seq2.fa -auto
-outfile seq1.seq2.needle
```

- dreg: regular expression search of a nucleotide sequence

```
dreg -sequence mySeq.tfa -pattern
GGAT[TC]TAA -outfile mySeq_dreg.txt
```

Shell script example

```
#!/bin/csh
# alignSeqs.csh: align a pair of sequences

# Check to make sure you get two arguments (sequence
   files)
if ($#argv != 2) then
   echo "Usage: $0 seq1 seq2"; exit 1
endif

# Local alignment
set localOut=$1.$2.water.out
water $1 $2 -auto -outfile $localOut
echo Wrote local alignment to $localOut

# Global alignment
set globalOut=$1.$2.needle.out
needle $1 $2 -auto -outfile $globalOut
echo Wrote global alignment to $globalOut
```

Some other helpful commands

- rm: remove (delete) files ex: **`rm myOldfile`**
- cat: concatenate files

  `ex: cat *.seq > all_seq.tfa`
- alias: create your own command shortcuts

  `ex: alias myblastx blastall -p blastx -d nr`
- find: find a lost file (ex: look for files with the .fa extension)

  `ex: find . -name \*.fa`
- diff; comm: compare files or lists
- sort: sort (alphabetically/numerically) lines in a file
- uniq: get list of non-redundant lines
- grep: search a file for a text pattern
- tar: combine files together for storage or transfer
- sftp: transfer files between machines
- gzip & gunzip: compress or uncompress a file

Summary

- Why Unix?
- The Unix operating system
- Files and directories
- Ten required commands
- Input/output and command pipelines
- X windows, EMBOSS, and shell scripts

Exercises

- compress, move, and uncompress sequence files
- make a multiple sequence file
- create a BLAST database
- run BLAST on your database
- extract a sequence from the database