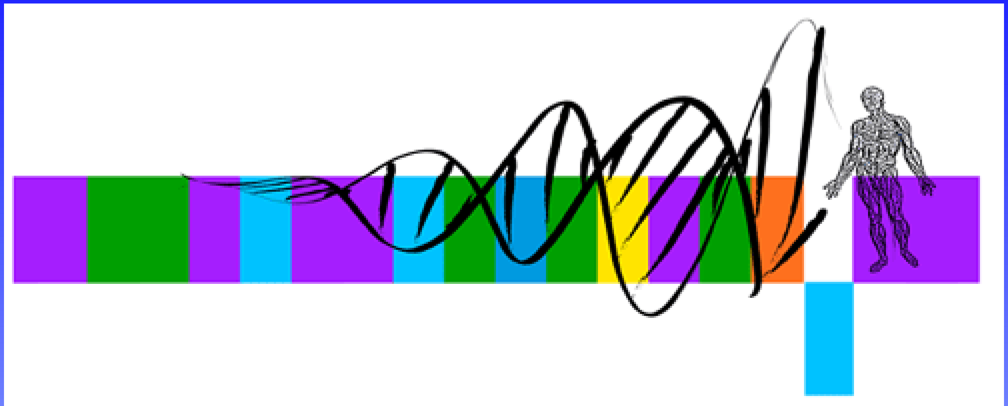

Unix, Perl and BioPerl

I: Introduction to Unix for Bioinformatics

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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

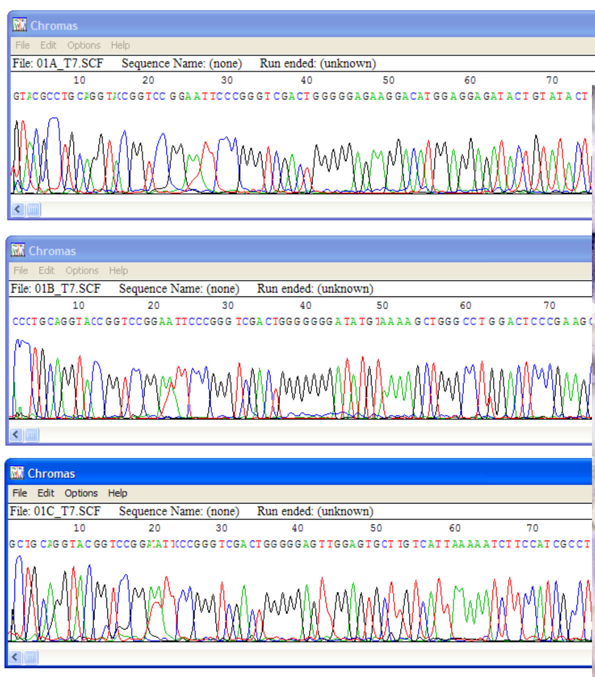
Objectives

- 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

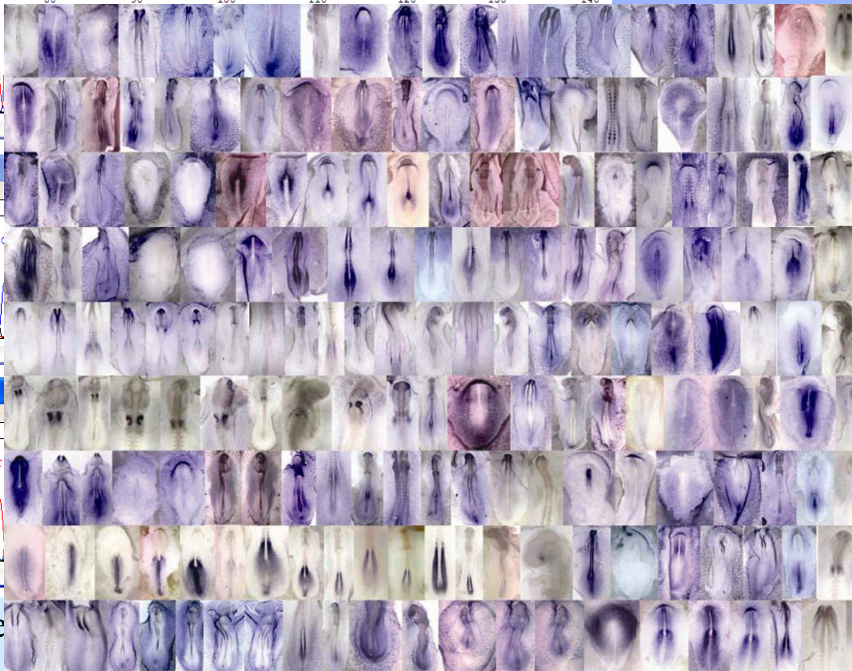
```
>A01_T3 | GEISHA | Gallus gallus | 496 nt | 77:572
ATCAAAGGCTTTACCGACAAACATCATTTGCACAATTAGTTGTTGGACAGGAGGGAGGACACCCGAGGACATGTAGGCTCGAGCCATAGTGTTGCCAAGGCTCTC
CCTGTTTGTTCCTTGGGTGAGCTGAGCCAACAGCTCTCCCTGCCCTCAGGAAGGCAGCAGTGGTGACAGGCACTCTATGGGGACTAACAGGAGGGGGTGGTTGTG
GTGACCTCGGAGCAGGCAGCATCTCACCCATCACTCACACTGCAGACAGCATCACTGTGAAGGCCTACAGATACTGCAGTGTGGGTCACAAAAGCATCCACTGGC
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
```



```
File: 01A_T7.SCF    Sequence Name: (none)    Run ended: (unknown)
        10        20        30        40        50        60        70
GTACGCCTGCAGGTACCGGTCCGGAATTCCCGGGTCGACTGGGGGAGAAGGACATGGAGGAGATACTGTATACT
```

```
File: 01B_T7.SCF    Sequence Name: (none)    Run ended: (unknown)
        10        20        30        40        50        60        70
CCCTGCAGGTACCGGTCCGGAATTCCCGGGTCGACTGGGGGGGATATGTAAAAGCTGGGCCTGGACTCCCGAAG
```

```
File: 01C_T7.SCF    Sequence Name: (none)    Run ended: (unknown)
        10        20        30        40        50        60        70
GCTGCAGGTACGGTCCGGAATTCCGGGTCGACTGGGGGAGTTGGAGTGCTTGTCATTAAAAATCTTCCATCGCCT
```



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

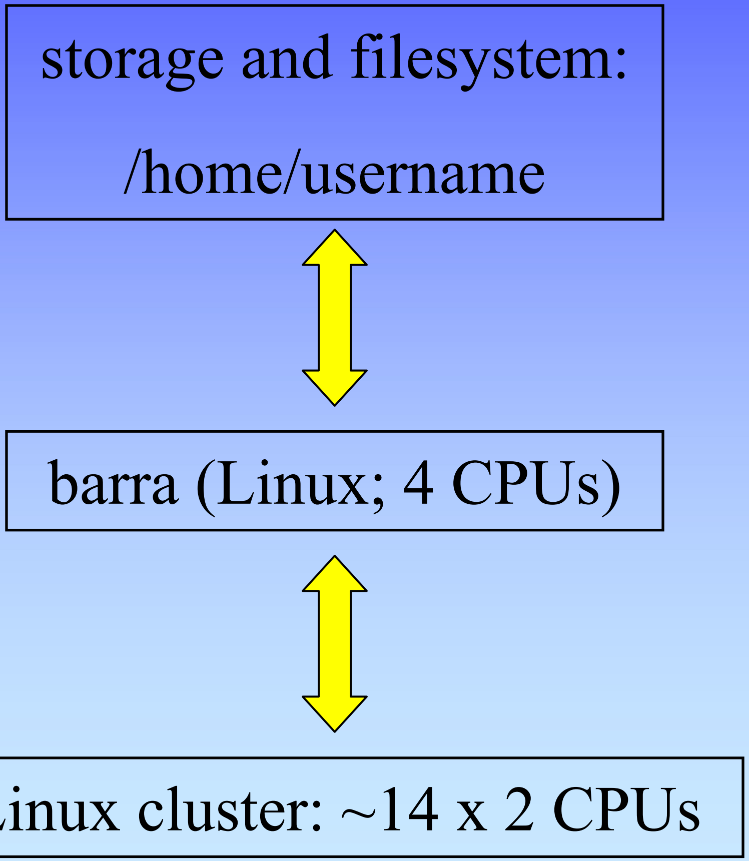
Training

hebrides

(Solaris; 4 CPUs)

with storage and filesystem /home/username

Research



storage and filesystem:

/home/username

barra (Linux; 4 CPUs)

Linux cluster: ~14 x 2 CPUs

Logging in

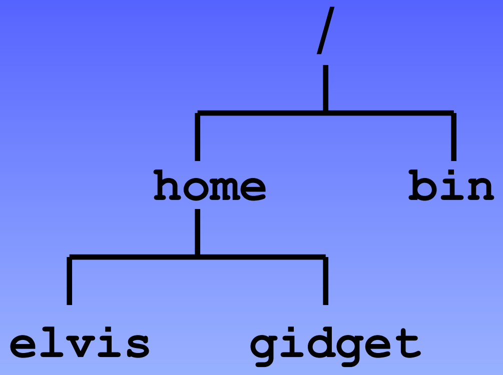
- ssh (secure shell; for encrypted data flow)
  ```
  ssh -l user_name hebrides.wi.mit.edu
  ```

- 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.tfa
```

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

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

1 = execute only

2 = write only

3 = x + w

4 = read only

5 = r + x

6 = r + 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`

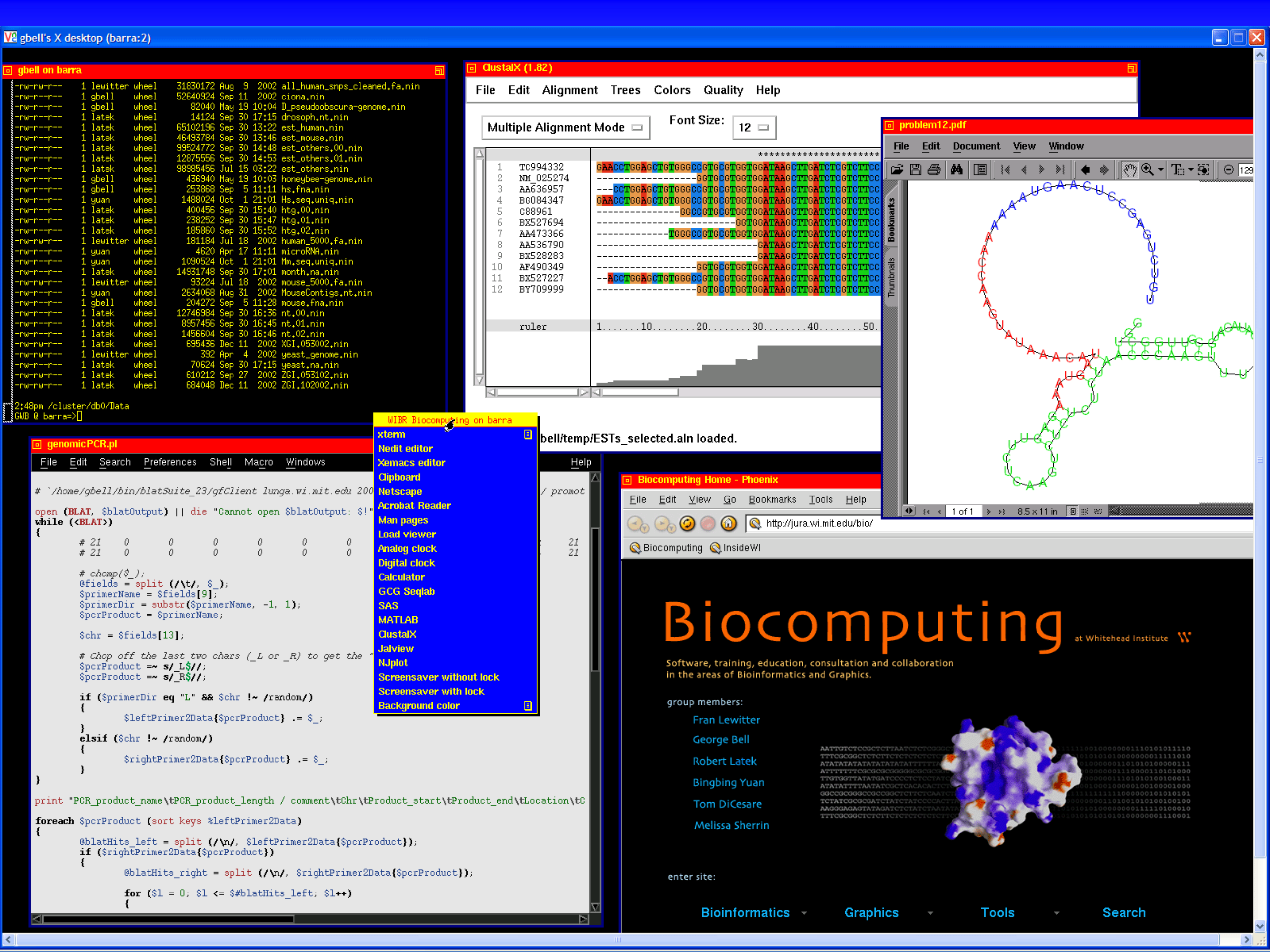
Text editors

- 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



```
-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=>
```

```
                                       ***********************
 1    TC994332   GAACCTGGAGCTGTGGGCCGTGCGTGGTGGATAAGCTTGATCTCGTCTTCC
 2    NM_025274  ------------------GGTGCGTGGTGGATAAGCTTGATCTCGTCTTCC
 3    AA636957   ---CCTGGAGCTGTGGGCCGTGCGTGGTGGATAAGCTTGATCTCGTCTTCC
 4    BG084347   GAACCTGGAGCTGTGGGCCGTGCGTGGTGGATAAGCTTGATCTCGTCTTCC
 5    C88961     ---------------GGCCGTGCGTGGTGGATAAGCTTGATCTCGTCTTCC
 6    BX527694   -------------------------GGTGGATAAGCTTGATCTCGTCTTCC
 7    AA473366   -------------TGGGCCGTGCGTGGTGGATAAGCTTGATCTCGTCTTCC
 8    AA536790   -----------------------------GATAAGCTTGATCTCGTCTTCC
 9    BX528283   -----------------------------GATAAGCTTGATCTCGTCTTCC
10    AF490349   ------------------GGTGCGTGGTGGATAAGCTTGATCTCGTCTTCC
11    BX527227   --ACCTGGAGCTGTGGGCCGTGCGTGGTGGATAAGCTTGATCTCGTCTTCC
12    BY709999   ------------------GGTGCGTGGTGGATAAGCTTGATCTCGTCTTCC

      ruler      1.......10........20........30........40........50.
```

bell/temp/ESTs_selected.aln loaded.

```
# `/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 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