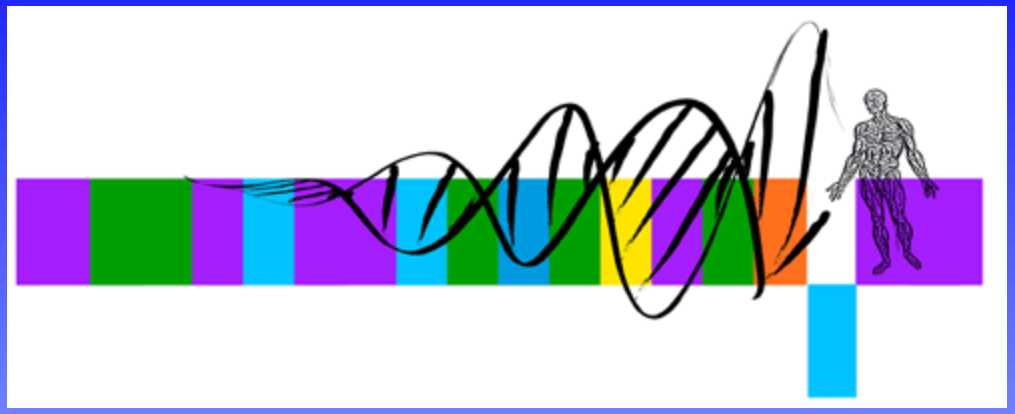

Unix, Perl and BioPerl

I: Introduction to Unix for Bioinformatics

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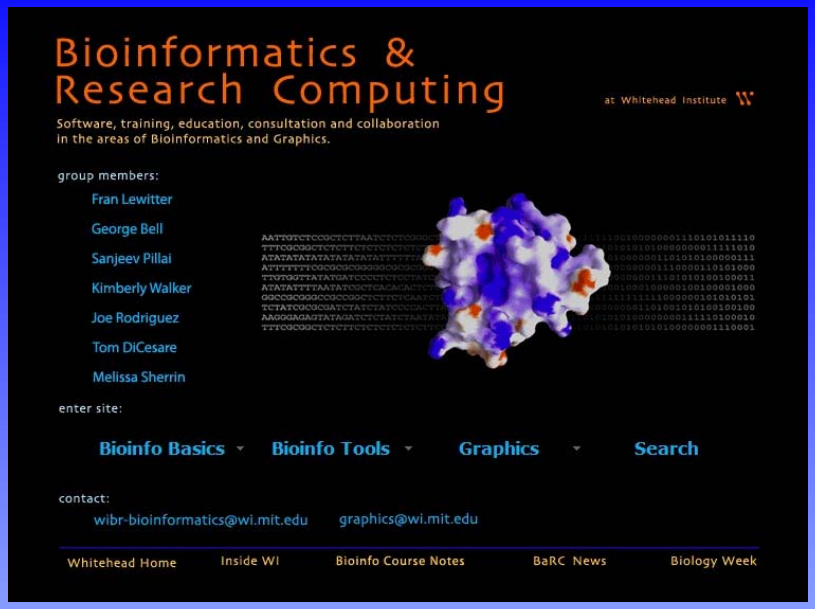
WIBR Bioinformatics and Research Computing



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
  - Use bioinformatics tools to analyze research data
  - Build new bioinformatics tools
  - 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

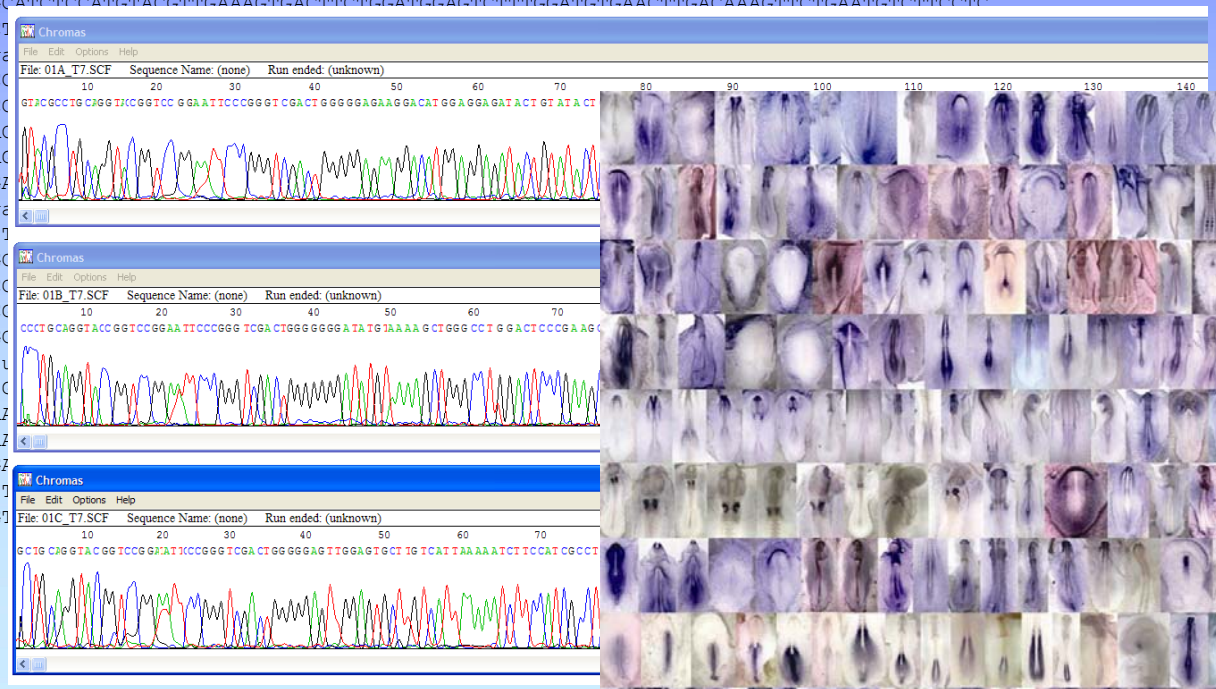
Objectives

- 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

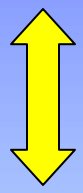
(Linux; 2 CPUs)

with storage and filesystem /home/username

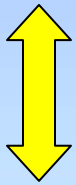
Research

storage and filesystem:

/home/username



barra (Linux; 4 CPUs)



Linux cluster: ~60 x 2 CPUs

Logging in

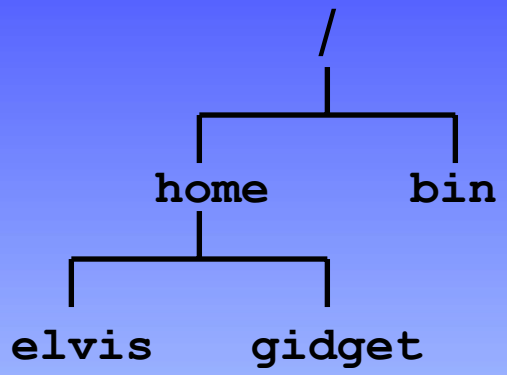
- ssh (secure shell; for encrypted data flow)
  `ssh -l user_name hebrides.wi.mit.edu`

- **`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.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	**cd**	**mv**
ls	**chmod**	**more**
rm		

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

Input/output redirection

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

  `command < inputFile > outputFile`
- input example

  `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

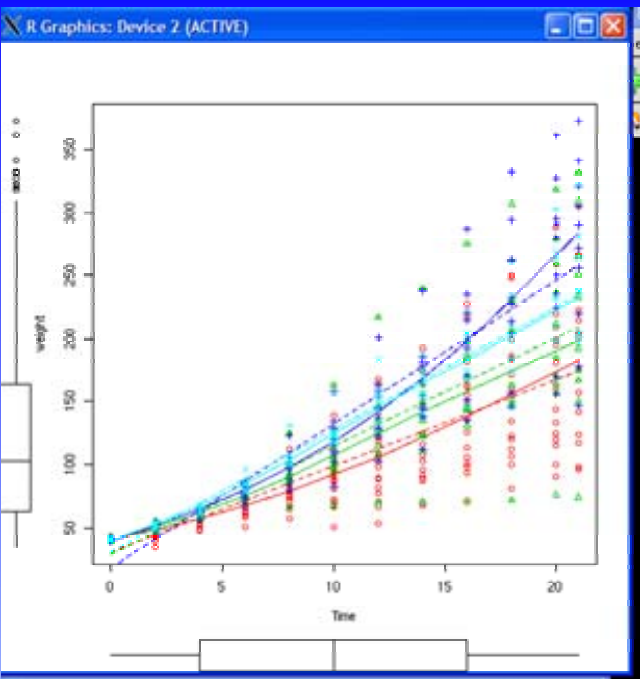
- emacs, vi (powerful but unfriendly at first); pico
- nedit, xemacs (easier; X windows only)
- desktop text editors (BBEdit; TextPad) + sftp

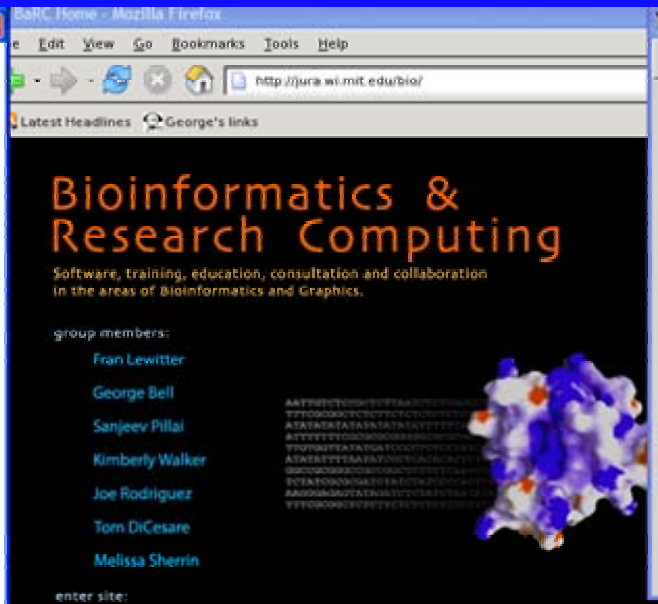
Supplementary information

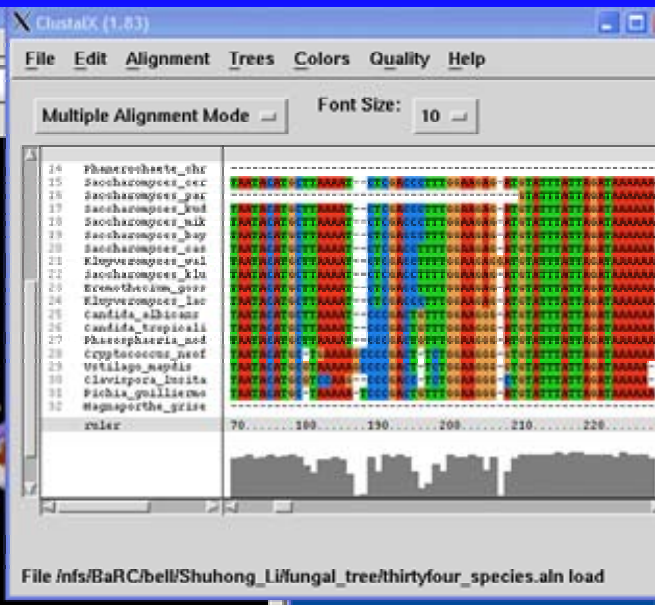
X Windows

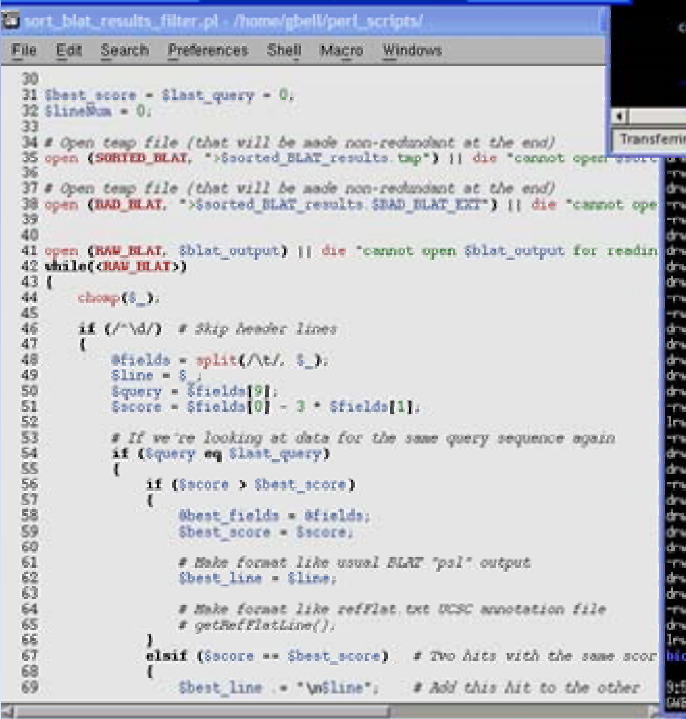
- method for running Unix graphical applications
- still allows for command-line operation
- see help pages for getting started
- some applications with extensive graphics:
  - EMBOSS
  - R
  - Matlab
  - ClustalX + njplot

- Requires a fast network/internet connection

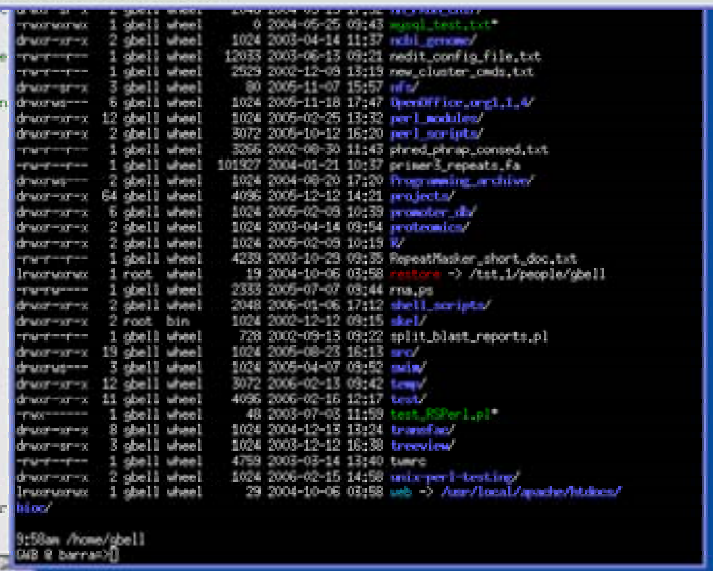


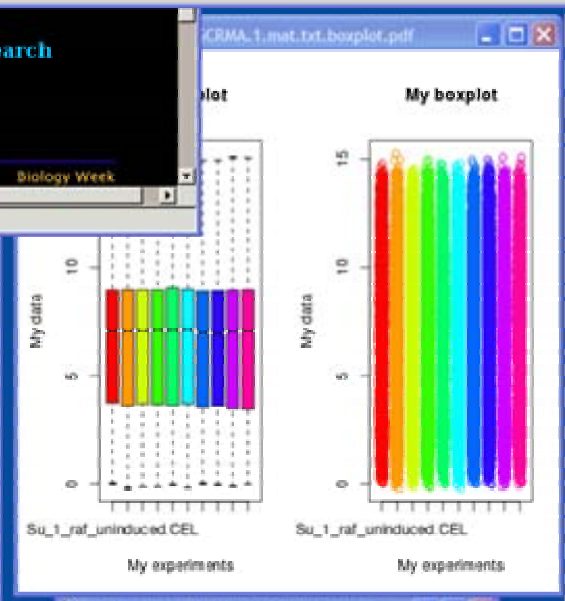


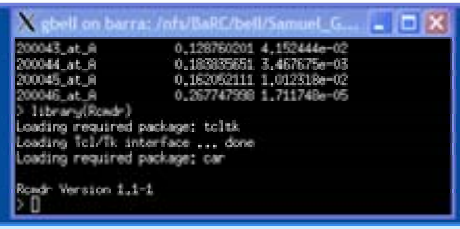





```
30
31 $best_score = $last_query = 0;
32 $lineNum = 0;
33
34 # Open temp file (that will be made non-redundant at the end)
35 open (SORTED_BLAT, ">$sorted_BLAT_results.tmp") || die "cannot open
36
37 # Open temp file (that will be made non-redundant at the end)
38 open (BAD_BLAT, ">$sorted_BLAT_results.$BAD_BLAT_EXT") || die "cannot ope
39
40
41 open (RAW_BLAT, $blat_output) || die "cannot open $blat_output for readin
42 while(<RAW_BLAT>)
43 {
44     chomp($_);
45
46     if (/^\d/)  # Skip header lines
47     {
48         @fields = split(/\t/, $_);
49         $line = $_;
50         $query = $fields[9];
51         $score = $fields[0] - 3 * $fields[1];
52
53         # If we're looking at data for the same query sequence again
54         if ($query eq $last_query)
55         {
56             if ($score > $best_score)
57             {
58                 @best_fields = @fields;
59                 $best_score = $score;
60
61                 # Make format like usual BLAT "psl" output
62                 $best_line = $line;
63
64                 # Make format like refFlat.txt UCSC annotation file
65                 # getRefFlatLine();
66             }
67             elsif ($score == $best_score)   # Two hits with the same scor
68             {
69                 $best_line .= "\n$line";    # Add this hit to the other
```







EMBOSS

- The European Molecular Biology Open Software Suite
- List of programs at http://emboss.sourceforge.net/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
- wget: download files from the web
- 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

Command-line interface:

- move and uncompress sequence files
- create a BLAST database and search it
- extract sequences from the database

Graphical (X Windows) interface:

- nedit, clustalx, njplot
- Image format conversion (**`ps2pdf, display`**)