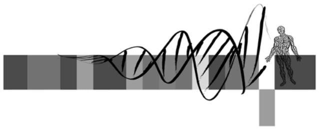

Bioinformatics for Biologists

Computational Methods I:

Genomic Resources and Unix

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Mammalian genome databases

- Organizing, analyzing, integrating, and presenting data
- Homes of major genome browsers:
  - NCBI
  - Ensembl
  - UCSC
- Which data/browsers best address your needs?
- Levels of use:
  1. Query remote database using web interface
  2. Write scripts to query remote database
  3. Install database locally and create queries however you want (SQL; Perl)

NCBI

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Genome

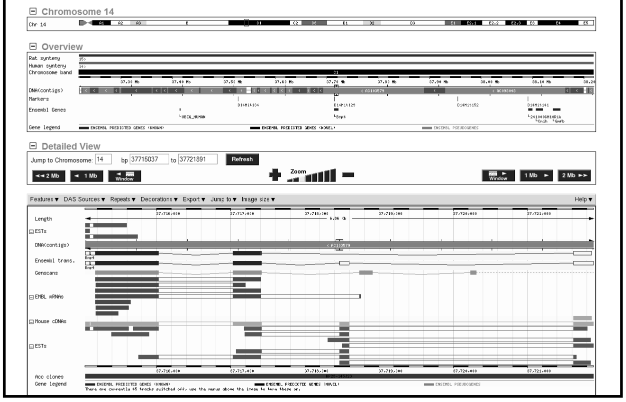
- Recent builds:
  - Human: July 2003 (Build 34)
  - Mouse: January 2003 (Build 30)
  - Rat: July 2003 (Build 2)
- Some ways to view the data:
  - Map View: browse a region of the genome
  - Evidence Viewer: see data on a gene model
  - Model Maker: create a gene model from data

Ensembl:

http://www.ensembl.org/

- A joint project: EMBL-EBI and the Sanger Institute
- Automated system for genome annotation: prediction + confirmation
- Genome-centric gene sequences
- Genes, exons, transcripts, and proteins
- Many data and display options
- Large analyses:
  - EnsMart
  - Download desired data tables (MySQL)

Ensembl ContigView



Jump to Chromosome: 14 bp 37715037 to 37721891

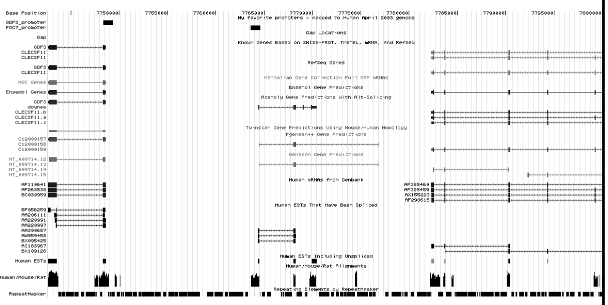
There are currently 45 tracks switched off, use the menus above the image to turn these on.

UCSC Genome Informatics:

http://genome.ucsc.edu/

- One view: alignment of data to genome
- BLAT: rapid alignment of cDNA to genome
- Many data and display options
- Easy to add custom annotation tracks
- Large analyses:
  - Table Browser
  - Download desired data tables (MySQL)

UCSC Genome Browser



Introduction to Unix

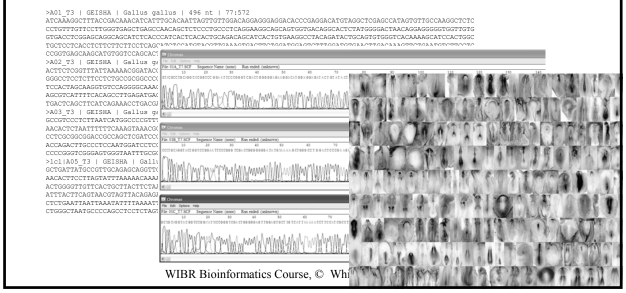
- Why Unix?
- The Unix operating system
- Files and directories
- Ten required commands
- Input/output and command pipelines
- Supplementary information
  - X windows
  - EMBOSS
  - Shell scripts

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
TGCTCCTCACCTCTTCTTCTTCCTCAG
CCGGTGAGCAAGCATGTGGTCCAGCACT
>A02_T3 | GEISHA | Gallus ga
ACTTCTCGGTTTATTAAAAACGGATAC
GGGCCTCCTCTTCCTCTGCCGCGGCCC
TCCACTAGCAAGGTGTCCAGGGGCAAA
AGCGTCATTTTCACAGCCTTGAGATGA
TGACTCAGCTTCATCAGAAACCTGACG
>A03_T3 | GEISHA | Gallus ga
GCCGTCCCTCTTAATCATGGCCCCGTT
AACACTCTAATTTTTTCAAAGTAAACG
CCTCGCGGCGGACCGCCAGCTCGATCC
ACCAGACTTGCCCTCCAATGGATCCTC
CCCCGGGTCGGGAGTGGGTAATTTGCG
>lcl|A05_T3 | GEISHA | Gallu
GCTGATTATGCCGTTGCAGAGCAGGTT
AACACTTCCTTAGTATTTAAAAACAAA
ACTGGGGTTGTTCACTGCTTACTTCTA
ATTTACTTCAGTAACGTAGTTACAGAG
CTCTGAATTAATTAAATATTTTAAAAT
CTGGGCTAATGCCCCAGCCTCCTCTAG
```

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, 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

Logging in

- ssh (secure shell; for encrypted data flow)

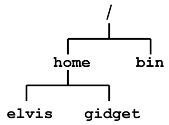
```
ssh –l user_name hebrides.wi.mit.edu
```

- 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')
- 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 new_data old_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
/usr/people/elvis
```

- **What's here?**

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

```
elvis@hebrides [3]% ls –a
.     .cshrc      A01.tfa
..    .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 *.fasta | wc -l`
  ex2: `head -1 *.fasta | grep '^>' | 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***

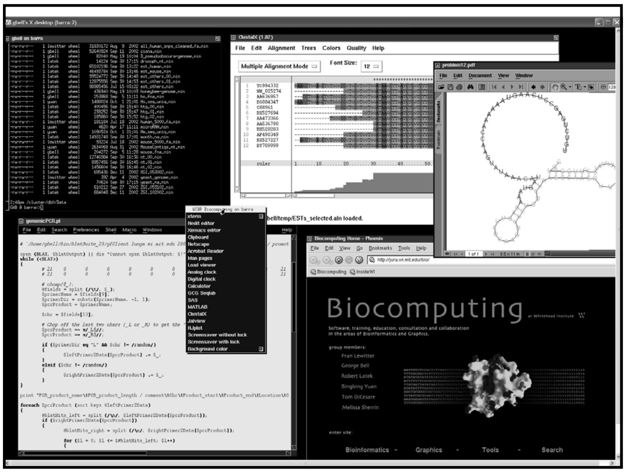
Text editors

- emacs, vi (powerful but unfriendly at first); pico
- xemacs, nedit (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
- 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   12746384 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:>
```

```
# `/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++)
                {
```

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
- 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

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

Demo on the web

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