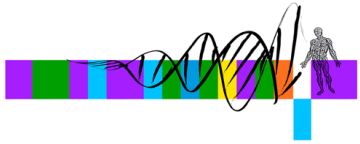

Unix, Perl and Python

Introduction to Unix and LSF

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Question

- I found 100 genes from de novo assembly, I want to quickly find out how many of them are potentially functional.
  - We can blast them against known protein databases.
  - Can we get an answer within one hour?

Outline

UNIX

1. About files/folders
2. Commonly used UNIX commands
3. Very useful bioinformatics commands

⇩

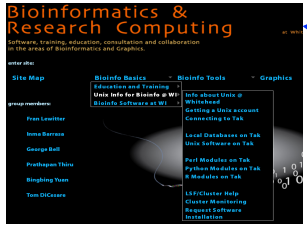
LSF (Load Sharing System)

Why Unix?

- Many repetitive analyses or tasks can be easily automated
- Some computer programs only run on the Unix operating system.
- TAK (our Unix server): lots of software and databases already installed or downloaded.
- Multiple remote users have access to the Unix at the same time.

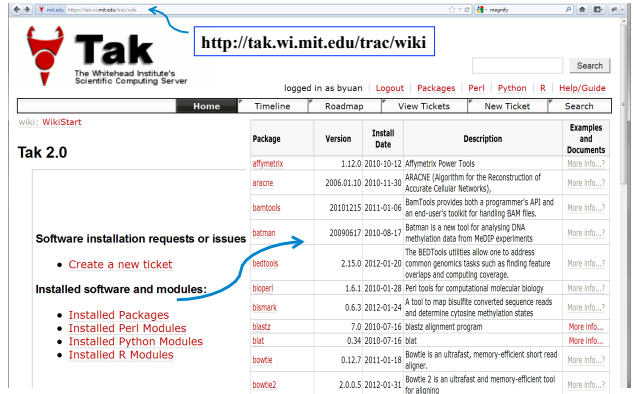
Where can UNIX be used?

- Mac computers
  Come with Unix
- Windows computers: Install Cygwin
- Dedicated Unix server
  "tak", the Whitehead Scientific Linux server



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

What is on tak?



Connect to tak with X Window

- Macs:
  1. Access to Terminal: Go => Utilities => Terminal
  2. log in to tak:

  `ssh -Y userName@tak`

  or

  `ssh -X userName@tak`

- Windows:
  1. Launch X Window Server: Xming
  2. Connect to tak with Secure Shell client: PuTTY

What is in the folder ?

List all files/directories

`ls` [only show names]

`ls -l` [long listing: show other information too]

```
byuan@tak ~/unix_2012$ ls
blast_seqs.sh*  seq.fa  temp/

byuan@tak ~/unix_2012$ ls -l
-rwxr--r--   1 byuan barc   1148   2012-03-25 10:05 blast_seqs.sh*
-rw-r--r--   1 byuan barc 150150 2012-03-25 10:05 seq.fa
drwxrwsr-x 2 byuan barc   4096   2012-03-25 10:06 results/
```

Who can read, edit and execute files?

Error: permission denied

- Mode: read, write, or execute files?
- Who: user (u), group (g), others (o), everybody (a)?

```
-rw-r--r-- byuan barc foo.pl
chmod u+x foo.pl
-rwxr--r-- byuan barc foo.pl
```

Allow user to execute script

```
-rw-r--r-- byuan barc document.txt
chmod g+w document.txt
-rw-rw-r-- byuan barc document.txt
```

Allow group to edit file

```
-rw-r--r-- byuan barc private.txt
chmod go-r private.txt
-rw------- byuan barc private.txt
```

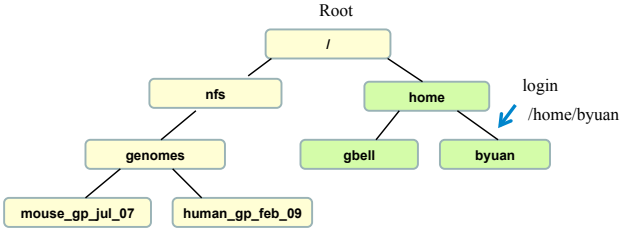
Only user can read/edit file

user group others

Where do you want to go?

Error: No such file or directory

- Print the working directory: **pwd**
- Change directories to where you want to go:
  cd dir
- Going up the hierarchy: **cd ..**
- Go back home: **cd or cd ~**
- Root: /
- Folders:
  - Lab: /nfs/ or /lab/
    e.g. /nfs/BaRC ⇔ WI-FILES1->BaRC
  - /nfs/BaRC_Public ⇔ WI-FILES1->BaRC_Public




```
byuan@tak ~$ pwd
/home/byuan
byuan@tak ~$ cd /nfs/genomes
byuan@tak /nfs/genomes$ cd ../../
byuan@tak /$ cd
byuan@tak ~$ _
```


How to organize files/folders ?

- Make a directory
  mkdir my_data
- Remove a directory (after emptying)
  rmdir my_data
- Move (rename) a file or directory
  mv oldFile newFile
- Copy a file
  cp oldFile newFileCopy
- Remove (delete) a file
  rm oldFile

Organize computational biology projects:
Plos Comp Bio. Jul;5(7):e1000424. Epub 2009

Combining commands

- In a pipeline of commands, the output of one command is used as input for the next
- Link commands with the "pipe" symbol: |

How many fasta files in the folder:

```
ls -l *.fa | wc -l
```

wc –l: count the number of lines

How many items mapped to chr15:

```
grep "chr15" myfile | wc -l
```

grep: print lines matching a pattern

Save files

- Defaults: stdin = keyboard; stdout = screen
- output examples

```
ls > file_name
```
(make new file)

```
ls >> file_name
```
(append to file)

```
ls foo >| file_name
```
(overwrite)

Read files

- **Display files on a page-by-page basis**

```
more file_name
```

↓ or ↑ move line by line Space: next page q: quit

- **Display first 2 lines of file:**

```
head -2 file_name
```

- **Display first 10 lines of file:**

```
head file_name
```

- **Display last 10 lines of file:**

```
tail file_name
```

- **Display the last line of file:**

```
tail -1 file_name
```

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LSF (Load Sharing System)

Concatenate files
cat

- Concatenate files

```
cat file1 file2 > bigFile
```

- Show file content at once

```
cat file
     A       it
     B       his
     D       her
```

- Show hidden characters with –A option

```
cat –A file      cat –A file
   A^Iit$           A^Iit^M$
   B^Ihis$          B^Ihis^M$
   D^Iher$          D^Iher^M$
```

From Excel

^I	TAB (\t)
$	end of line ($)
^M	carriage return(\r)

Print lines matching a pattern
grep

```
byuan@tak$ cat   FILE
chr19.fa    4126539   R
chr6.fa     81889764  R
Chr6.fa     77172493  R
byuan@tak$ grep -v 'chr19' FILE
chr6.fa     81889764  R
Chr6.fa     77172493  R
```

```
byuan@tak$ grep 'chr6' FILE
chr6.fa     81889764  R
byuan@tak$ grep -i 'chr6' FILE
chr6.fa     81889764  R
Chr6.fa     77172493  R
byuan@tak$ grep -n -i 'chr6' FILE
2:chr6.fa   81889764  R
3:Chr6.fa   77172493  R
```

-v	Select non-matching lines
-i	Ignore case
-n	Print line number

Sort lines of text files:
sort

```
cat FILE
chr6    34314346   F
chr6    52151626   R
chr6    81889764   R
chr6    52151626   R
```

```
cat geneFile
geneA   chr6   34314346  F
geneB   chr8   52151626  R
geneC   chr6   11889764  R
```

```
sort FILE
chr6    34314346   F
chr6    52151626   R
chr6    52151626   R
chr6    81889764   R
```

```
# sort by chromosome and by genomic location
sort -k 2,2 -k 3,3n geneFile
geneC   chr6   11889764  R
geneA   chr6   34314346  F
geneB   chr8   52151626  R
```

```
sort –u FILE
chr6    34314346   F
chr6    52151626   R
chr6    81889764   R
```

-n	numerical sort
-r	reverse the result of comparisons
-k pos1,pos2	Start a key at pos1, end it at pos2
-u	unique

cut sections from each line of files
cut

```
cat sample.gtf
chr16  mm9_refGene  exon  8513522 8621658 0.000000  +  .  gene_id "Abat"; transcript_id "NM_172961"
chr16  mm9_refGene  exon  8513522 8621658 0.000000  +  .  gene_id "Abat"; transcript_id "NM_001170978"
chr1   mm9_refGene  exon  134212715  134230065  0.000000  +  .  gene_id "Nuak2"; transcript_id "NM_028778"
# show hidden characters
cat -A sample.gtf
chr16^Imm9_refGene^Iexon^I8513522^I8621658^I0.000000^I+^I.^Igene_id "Abat"; transcript_id "NM_172961"$
chr16^Imm9_refGene^Iexon^I8513522^I8621658^I0.000000^I+^I.^Igene_id "Abat"; transcript_id "NM_001170978"$
chr1^Imm9_refGene^Iexon^I134212715^I134230065^I0.000000^I+^I.^Igene_id "Nuak2"; transcript_id "NM_028778"$
# last field separated by tab
cut -f9 sample.gtf
gene_id "Abat"; transcript_id "NM_001170978"
gene_id "Abat"; transcript_id "NM_172961"
gene_id "Nuak2"; transcript_id "NM_028778"
# gene names:
cut -d " " -f2 sample.gtf
"Abat";
"Abat";
"Nuak2";
#  unique gene names
 cut -d " " -f2 sample.gtf | sort -u
"Abat";
"Nuak2";
```

-f	output only these fields
-d	field delimiter Default: TAB

report or omit repeated lines

uniq

```
cat genes.txt
Abat	NM_172961
Abat	NM_001170978
Nuak2	NM_028778
```

```
cut -f1 genes.txt
Abat
Abat
Nuak2
```

```
# How many transcripts each gene has ?
cut -f1 genes.txt | uniq -c
   2 Abat
   1 Nuak2
# Which genes have multiple transcripts?
cut -f1 genes.txt | uniq -d
Abat

# Which genes have only one transcript?
cut -f1 genes.txt | uniq -u
Nuak2
```

Note: run `sort` before `uniq`

Downloading files from the web

- **Directly save to tak from web:**

```
wget ftp://ftp.ncbi.nih.gov/pub/geo/...GSM537962%2ECEL%2Egz
```

- **Decompress files:**

```
gunzip file.gzip
tar -xvf file.tar
tar -xzf file.tar.gz
tar -xzf /lab/solexa_public/xxx/s_6_sequence.txt.tar.gz -O > s_6_sequence
```

-x: extract files from archive.
-f: specifies filename / tarball name.
-v: Verbose (show progress while extracting files).
-z: filter the archive through gzip, use to decompress .gz files.
-O: extract files to standard output

Notes

- Use up arrow, down arrow to re-use previous commands
- CTRL-C: stop process that are running
- Auto-complete with TAB (filename)
- When reading files/documents:

  ↓ or ↑ move line by line space: next page q: quit
- One-line description of command: **whatis**

  ```
  whatis mv
  ```
- To get help (manual) command: **man**

  ```
  man ls
  ```
- Avoid filenames with spaces
  - If necessary to use, refer to with quotes:

  ```
  "My dissertation version 1 .txt"
  ```
- Case sensitive: directories/files, commands

Outline

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LSF (Load Sharing System)

Run blast locally

blastall

Common Switches:

option	description	default
-p	Program Name	
-d	Database	nr
-i	Query File	stdin
-e	Expectation Value	10
-m	Alignment view options	
-o	output	stdout

Column descriptions for BLAST tabular output file with –m 8 option

1. query id
2. subject id
3. percent id
4. alignment length
5. number of mismatches
6. number of gap openings
7. query start
8. query end
9. subject start
10. subject end
11. expect value
12. bit score

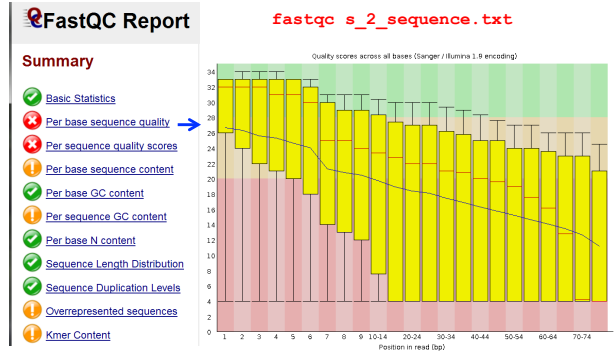
```
blastall -p blastx -e 1e-10 -m 8 -i transcripts.fa -d hs.faa -o hs_blastx.txt
```

```
TCONS_00035902  gi|154937380|ref|NP_001094348.1|    100.00  16   0   0   480   527   345   360   6e-55  36.2
TCONS_00039543  gi|256418948|ref|NP_009084.2|       65.00  20   7   0   2726  2785  1244  1263  0.0    31.6
```

More information on blastall
http://www.ncbi.nlm.nih.gov/staff/tao/URLAPI/blastall/blastall_node3.html
List of local databases:
http://iona.wi.mit.edu/bio/databases/list.php

Check the quality of Illumina reads

FastQC http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/




```
fastqc s_2_sequence.txt
```


Remove reads with low quality

fastq_quality_filter

```
fastq_quality_filter -h
```

[-q N] = Minimum quality score to keep.
[-p N] = Minimum percent of bases that must have [-q] quality
[-i INFILE] = FASTA/Q input file. default is STDIN.
[-o OUTFILE] = FASTA/Q output file. default is STDOUT.

```
fastq_quality_filter -q 20 -p 75 -i myFile.fq -o myFile.trimmed.fq
```

FASTX Toolkit: http://hannonlab.cshl.edu/fastx_toolkit/

Genomic features overlap with each other ?

intersectBed

```
intersectBed
```

Usage: bedtools intersect [OPTIONS] -a <bed/gff/vcf> -b <bed/gff/vcf>

Options:
-abam The A input file is in BAM format. Output will be BAM as well.
-ubam Write uncompressed BAM output. Default writes compressed BAM.
-s Require same strandedness. That is, only report hits in B that overlap A on the _same_ strand.
- By default, overlaps are reported without respect to strand.

```
intersectBed -a genes.bed -b peaks.bed > Overlapped.bed
```

Bedtools: http://code.google.com/p/bedtools/

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⇩

LSF (Load Sharing System)

LSF (Load Sharing Facility)

- Jobs takes a long time to finish
- Speed: multiple jobs running at same time

Hosts

https://tak.wi.mit.edu/trac/

Software installation requests or issues:

- Create a new ticket

Installed software and modules:

- Installed Packages
- Installed Perl Modules
- Installed Python Modules
- Installed R Modules

LSF Cluster Activity

User Name	Jobs Running	Jobs Pending
byuan	1	0
ibarras	21	0
jwnam	1	1
kmck	1	1
orlando	1	0
sgupta	2	0
Totals:	27	2

Last updated: Tue Jan 17 13:30:01 EST 2012

Host Available Host Closed Host Unavailable

Host	Status	Max Jobs	Running	CPU Cores	CPU Speed	Memory	Uptime	Load	System Info
it-c01b04	Available	8	1	8	1.99 GHz	32 GB	179 days	1.02%	HP ProLiant BL460c G1
it-c01b05	Available	8	1	8	2.33 GHz	32 GB	213 days	1.59%	HP ProLiant BL460c G1
it-c01b06	Available	8	2	8	1.86 GHz	32 GB	213 days	2.08%	HP ProLiant BL460c G1
it-c01b07	Available	8	1	8	2.33 GHz	14 GB	66 days	1.20%	HP ProLiant BL460c G1
it-c01b08	Available	8	1	8	2.33 GHz	16 GB	213 days	1.17%	HP ProLiant BL460c G1
it-c01b09	Available	8	2	8	2.33 GHz	16 GB	213 days	2.04%	HP ProLiant BL460c G1
it-c01b10	Available	8	1	8	2.33 GHz	16 GB	213 days	1.70%	HP ProLiant BL460c G1
it-c01b11	Available	8	1	8	2.33 GHz	16 GB	213 days	1.90%	HP ProLiant BL460c G1
it-c01b12	Available	8	1	8	2.33 GHz	16 GB	213 days	1.15%	HP ProLiant BL460c G1
it-c01b13	Available	8	1	8	2.33 GHz	16 GB	213 days	1.06%	HP ProLiant BL460c G1
it-c01b14	Available	8	1	8	2.33 GHz	16 GB	213 days	1.06%	HP ProLiant BL460c G1
it-c01b15	Available	8	1	8	2.33 GHz	16 GB	213 days	1.28%	HP ProLiant BL460c G1
it-c05b02	Available	8	2	16	2.53 GHz	72 GB	209 days	2.25%	HP ProLiant BL460c G6
it-c05b03	Unavailable	1	0	16	2.53 GHz	72 GB	2 days	0.00%	HP ProLiant BL460c G6

submit jobs:
bsub

```
bsub  myscript.pl
bsub  "myscript.pl > result"
```

- Send error and standard output to files

```
bsub -e error_file -o std_file myscript.pl
```

- Send job to a host

```
bsub -m it-c01b08 myscript.pl
```

- Send notification to specified email

```
bsub -u foo@gmail.com myscript.pl
```

Check the job status
bjobs

```
byuan@tak ~$ bjobs
JOBID   USER    STAT  QUEUE      FROM_HOST   EXEC_HOST   JOB_NAME   SUBMIT_TIME
101853  byuan   RUN   normal     tak         it-c01b12   *apped.sam Mar 14 12:14
101873  byuan   RUN   normal     tak         it-c05b14   *apped.sam Mar 14 12:52

byuan@tak ~$ bjobs -l 101853
Job <101853>, User <byuan>, Project <default>, Status <RUN>, Queue <normal>, Job
    Priority <50>, Command <bowtie --best -k 2 -S /nfs/genomes/human_gp_feb_09/
    bowtie/hg19 H3K36me3.trimmed.fastq H3K36me3.mapped.sam>
Wed Mar 14 12:14:40: Submitted from host <tak>, CWD </lab/solexa_scratch/byuan> ;
 STACKLIMIT CORELIMIT OPENFILELIMIT
   8192 K       0 K    1024
Wed Mar 14 12:14:40: Started on <it-c01b12>, Execution Home </home/byuan>, Exec
                     ution CWD </lab/solexa_scratch/byuan>;
Wed Mar 14 13:24:37: Resource usage collected.
                     The CPU time used is 4125 seconds.
                     MEM: 2290 Mbytes;  SWAP: 2447 Mbytes;  NTHREAD: 4
                     PGID: 9086;  PIDs: 9086 9093 9095
 SCHEDULING PARAMETERS:
           r15s   rlm  r15m   ut      pg    io   ls    it    tmp    swp    mem
 loadSched   -     -     -     -       -     -    -     -     -      -      -
 loadStop    -     -     -     -       -     -    -     -     -      -      - st
```

Kill jobs:
bkill

```
bjobs
JOBID   USER    STAT  QUEUE      FROM_HOST   EXEC_HOST   JOB_NAME   SUBMIT_TIME
103889  byuan   RUN   normal     tak         it-c05b10   *med.fastq Mar 16 10:35
103890  byuan   RUN   normal     tak         it-c05b12   *leaned.fq Mar 16 10:37
103891  byuan   RUN   normal     tak         it-c05b12   *eaned2.fq Mar 16 10:37
bkill 103889
Job <103889> is being terminated
bjobs
JOBID   USER    STAT  QUEUE      FROM_HOST   EXEC_HOST   JOB_NAME   SUBMIT_TIME
103890  byuan   RUN   normal     tak         it-c05b12   *leaned.fq Mar 16 10:37
103891  byuan   RUN   normal     tak         it-c05b12   *eaned2.fq Mar 16 10:37
bkill 0
Job <103890> is being terminated
Job <103891> is being terminated
bjobs
No unfinished job found
```

Kill all jobs

commands

ls	**pwd**	**chmod**	**ln -s**
cp	**mv**	**rm**	**mkdir**
rmdir	**more**	**head**	**tail**
cat	**cut**	**gunzip**	**tar**
wget	**sort**	**uniq**	**wc -l**
grep	**whatis**	**man**	
bsub	**bjobs**	**bkill**	

blastall **fastQC**
fastq_quality_filter **intersectBed**

Further Reading

- BaRC: Getting Started with UNIX
  - http://iona.wi.mit.edu/bio/education/unix_intro.html
    - tak account, transfer files, X Windows, unix commands, video.
- Whitehead Linux cluster - LSF help
  - http://iona.wi.mit.edu/bio/bioinfo/docs/LSF_help.php
    - Popular commands, video, manuals
- UNIX Tutorial for Beginners
  - http://www.ee.surrey.ac.uk/Teaching/Unix/