Linux: Beyond the Basics

Bingbing Yuan and George Bell
BaRC Hot Topics – Oct. 2022
Bioinformatics and Research Computing
Whitehead Institute

http://barc.wi.mit.edu/hot_topics/

Whitehead Institute

Logging in to our Linux servers

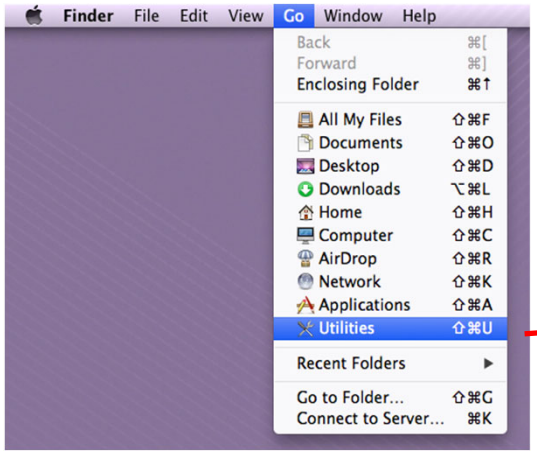
- Our main Linux servers are tak and fry
  fry has the most up-to-date software

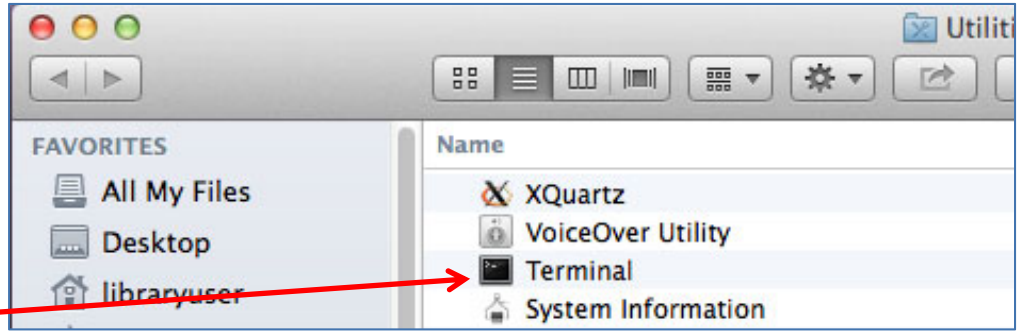
- Request a tak/fry account:
  https://element.wi.mit.edu/am/?f=unixRequest

- Connecting to tak or fry:
  http://bioinfo.wi.mit.edu/bio/software/unix/serverConnect.php

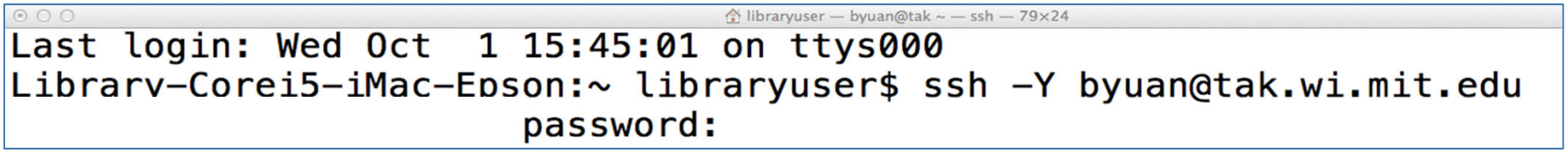
  - Windows:
    - MobaXterm
  - Mac
    - Access through Terminal

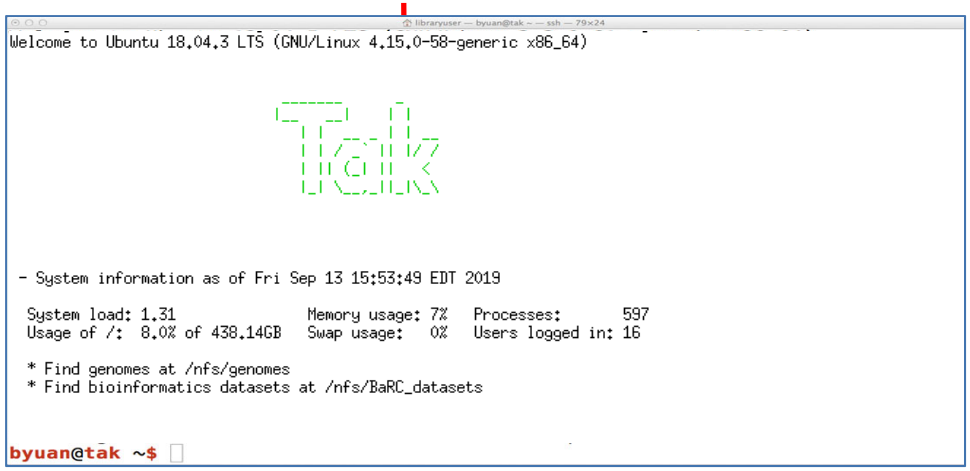
Log in to tak for Mac





ssh –Y username@tak.wi.mit.edu





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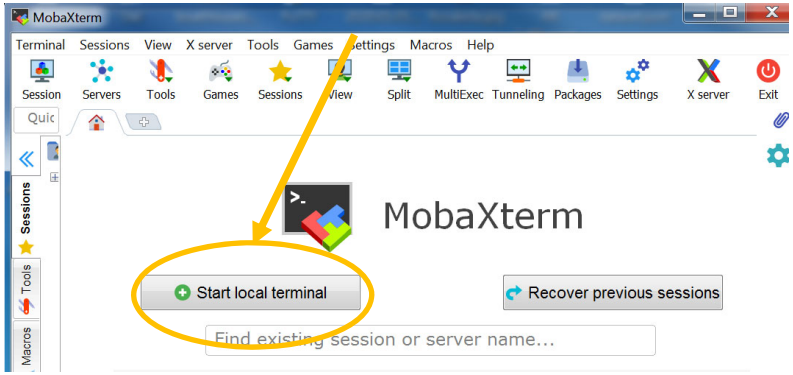
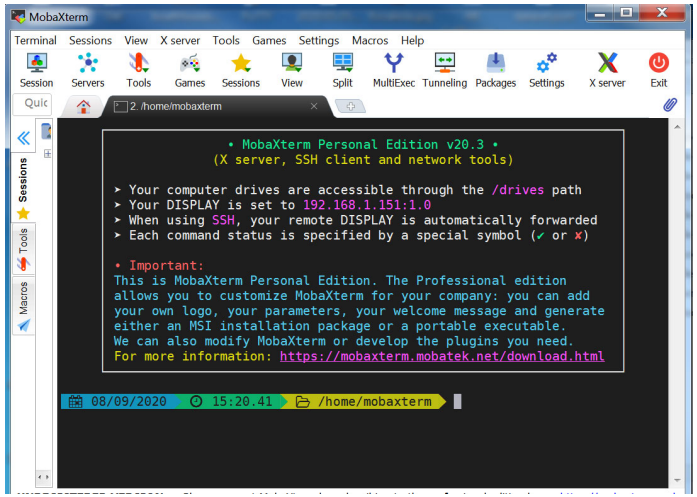
Connecting to tak from Windows

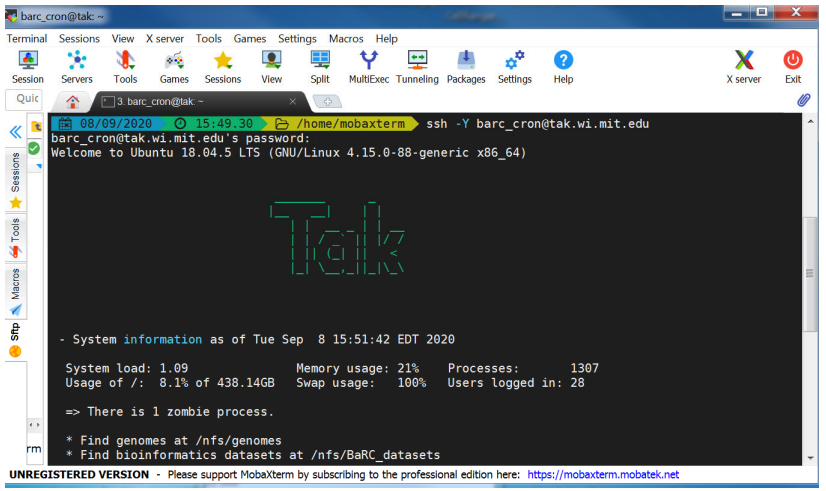
MobaXterm

1) Open MobaXterm

2) Click on the ”Start local terminal” button







3) Type:

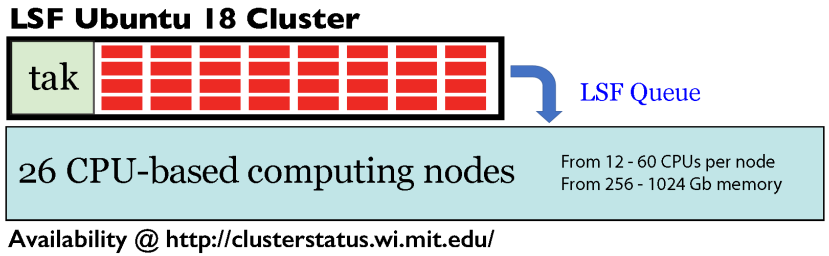
```
ssh -Y username@tak.wi.mit.edu
```

Note:
When you write the password you won’t see any characters being typed.

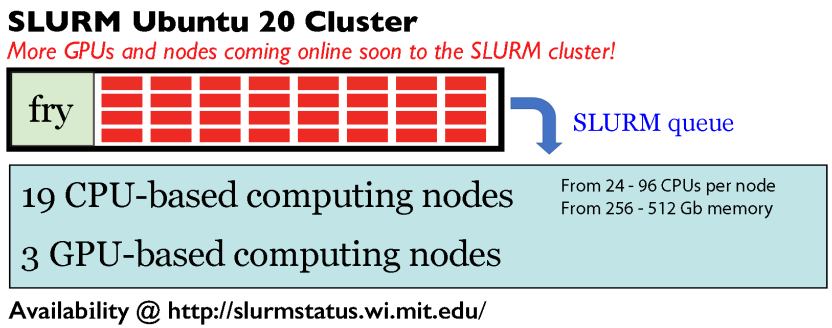
Sending big commands to the cluster

What does this command do?

```
# Send command from tak to lsf cluster
bsub "sleep 100; pwd; ls -l"
# Check in progress status
bjobs -w
bpeek <jobID>
# Read command output => check email
```




```
# Send command from fry to slurm cluster
sbatch --partition=20 --job-
name=HT_TEST --mem=4G --wrap
"sleep 100; pwd; ls -l"
# Check in-progress status
squeue
cat slurm-*out
# Get command output => read slurm-*out
```



Hot Topics website: http://barc.wi.mit.edu/education/hot_topics/

- After you login to tak, create a directory for the exercises within your home directory, and use it as your working directory

  $ mkdir Hot_Topics

  $ cd Hot_Topics

- Copy all files into your working directory

  $ cp -r /nfs/BaRC_training/Linux_Beyond/data_files/* .

- You should have the files below in your working directory:
- **datasets**/ Ensembl_info.txt exercise.txt foo.txt Gene_exp.txt HumanGenes PlusMinus3kb.bed peaks.bed sample1.txt exercise.pdf
  - You can check they're there with one of these commands:
    - **`ls`**
    - **`find`**

Linux Review: Commands

- **`command [arg1 arg2 … ] [input1 input2 … ]`**

$ sort -k2,3nr foo.tab

start end

-n or **-g**: -n is recommended, except for scientific notation or a leading '+'

-r: reverse order

$ cut -f1,5 foo.tab

$ cut -f1-5 foo.tab

-f: select only these fields
-f1,5: select 1st and 5th fields
-f1-5: select 1st, 2nd, 3rd, 4th, and 5th fields

$ wc -l foo.txt

How many lines are in this file?

Linux Review: Common Mistakes

- Case sensitive

`cd /nfs/Barc_Public` is different from **`cd /nfs/BaRC_Public`**

```
-bash: cd: /nfs/Barc_Public: No such file or directory
```

- Spaces may matter!

  `rm –f myFiles*` **vs** **`rm –f myFiles *`**

- Office applications can convert text to special characters that Linux won't understand
  - Smart quotes, dashes
  - Carriage return from DOS
    - Use fromdos to remove carriage return

Linux Review: Pipes

- Stream output of one command/program as input for another
  - Avoid intermediate file(s)
  - Merge multiple commands in to one long command

➢ $ cut -f1 myFile.txt | sort | uniq -c > uniqCounts.txt

↗ pipes ↗

What we will discuss today

- Aliases (to reduce typing)
- sed (for file manipulation)
- awk(to filter by column)
- join (merge files)
- groupBy and intersect from bedtools (not typical Linux)
- loops (one-line and with shell scripts)
- scripting (to streamline commands)

Aliases

- Add a one-word link to a longer command
- To get current aliases (from ~/.bashrc)

```
alias
```

- Create a new alias (examples)

```
alias sp='cd /lab/solexa_public/Lodish'
alias picard='java -jar /usr/local/share/picard-tools/picard.jar'
alias lr='ls -ltr'
```

- Make an alias permanent
  - Paste command(s) in ~/.bashrc

sed:

stream editor for filtering and transforming text

- Print lines 10 - 15:

```
$ sed -n '10,15p' bigFile > selectedLines.txt
```

- Delete 5 header lines at the beginning of a file:

```
$ sed '1,5d' file > fileNoHeader
```

- Remove all version numbers (eg: '.1') from the end of a list of sequence accessions: eg. NM_000035.2

```
$ sed 's/\.[0-9]\+//g' accsWithVersion > accsOnly
```

s: substitute *g: global modifier (change all)*

- Get good examples from “sed cheat sheet”:
  - https://www.pement.org/sed/sed1line.txt

Join files together

With Linux join

```
$ join -1 1 -2 2 --nocheck-order -t $'\t' sorted_File1 sortedFile2
```

Join files on the 1st field of FILE1 with the 2nd field of FILE2,

only showing the common lines.

FILE1 and FILE2 **must** be sorted on the join fields before running join

-t $'\t' : Both input and output use tab as separator

--nocheck-order: good for sorted files with header line.

Sorted sample tables to join:

Symbol	Heart	Skeletal Muscle	Skin	Smooth Muscle	Spinal cord
HHAT	8.15	7.7	5	6.55	6.4
INPP5D	19.65	5.95	4.55	5.25	14.5
NDUFA10	441.8	160.2	24.9	188.85	158.75
RPS6KA1	85.2	47.75	46.45	35.85	44.55
RYBP	20.45	13.05	11.95	20.7	17.75
SLC16A1	15.45	20.45	12.2	248.35	27.15

Ensembl Gene ID	Symbol
ENSG00000252303	RNU6-280P
ENSG00000280584	OBP2B
ENSG00000280680	HHAT
ENSG00000280775	RNA5SP136
ENSG00000280820	LCN1P1
ENSG00000280963	SERTAD4-AS1

Symbol	Heart	Skeletal Muscle	Skin	Smooth Muscle	Spinal cord	Ensembl Gene ID
HHAT	8.15	7.7	5	6.55	6.4	ENSG00000280680

Regular Expressions

- A sequence of characters defining a search pattern
- Powerful, but syntax is often non-intuitive
- Examples

  List all txt files: `ls *.txt`

  Replace CHR with Chr at the beginning of each line:

  ```
  $ sed 's/^CHR/Chr/' myFile.txt
  ```

  Delete a dot followed by one or more numbers

  ```
  $ sed 's/\.[0-9]\+//g' myFile.txt
  ```

	Matches
.	All characters
*	Zero or more; wildcard
+	One or more
?	One
^	Beginning of a line
$	End of a line
[ab]	Any character in brackets

- Note: regular expression syntax may slightly differ between sed, awk, Linux shell, and Perl
  - Ex: \+ in sed is equivalent to + in Perl

awk

- A simple programing language to process files
- Good for filtering and manipulating multiple-column files
- “awk” comes from the original authors:

  Alfred V. Aho, Peter J. Weinberger, Brian W. Kernighan

awk

- By default, awk splits each line by spaces

- Print the 2nd and 1st fields of the file:

```
$ awk ' { print $2"\t"$1 } ' foo.tab
```

- Convert sequences from tab delimited format to fasta format:

```
$ head -1 foo.tab
Seq1    ACTGCATCAC
$ awk ' { print ">" $1 "\n" $2 }' foo.tab  >  foo.fa
$ head -2 foo.fa
>Seq1
ACGCATCAC
```

awk: field separator

- Issues with default separator (white space)
  - one field is gene description with multiple words
  - consecutive empty cells
- To use tab as the separator:

```
$ awk -F "\t" '{ print NF }' foo.txt
```

or

```
$ awk 'BEGIN {FS="\t"} { print NF }' foo.txt
```

BEGIN: action before read input
NF: number of fields in the current record
FS: input field separator
OFS: output field separator
END: action after read input

Character	Description
\n	newline
\r	carriage return
\t	horizontal tab

awk: arithmetic operations

Add average values of 4th and 5th fields to the file:
$ awk '{ print $0 "\t" ($4+$5)/2 }' foo.tab

$0: all fields

Operator	Description
+	Addition
-	Subtraction
*	Multiplication
/	Division
%	Modulo
^	Exponentiation
**	Exponentiation

awk: making comparisons

Print out records if values in 4th or 5th field are above 4:

```
$ awk '{ if( $4>4 || $5>4 ) print $0 } ' foo.tab
```

Sequence	Description
>	Greater than
<	Less than
<=	Less than or equal to
>=	Greater than or equal to
==	Equal to
!=	Not equal to
~	Matches
!~	Does not match
||	Logical OR
&&	Logical AND

More awk examples

- Conditional statements:

Display expression levels for the gene NANOG:

```
$ awk '{ if(/NANOG/) print $0 }'  foo.txt
```

or

```
$ awk '/NANOG/ { print $0 } ' foo.txt
```

or

```
$ awk '/NANOG/' foo.txt
```

Add line number to the above output:

```
$ awk '/NANOG/ { print NR"\t"$0 }'  foo.txt
```

NR: line number of the current row

- Looping:

Calculate the average expression (4th, 5th and 6th fields in this case) for each transcript

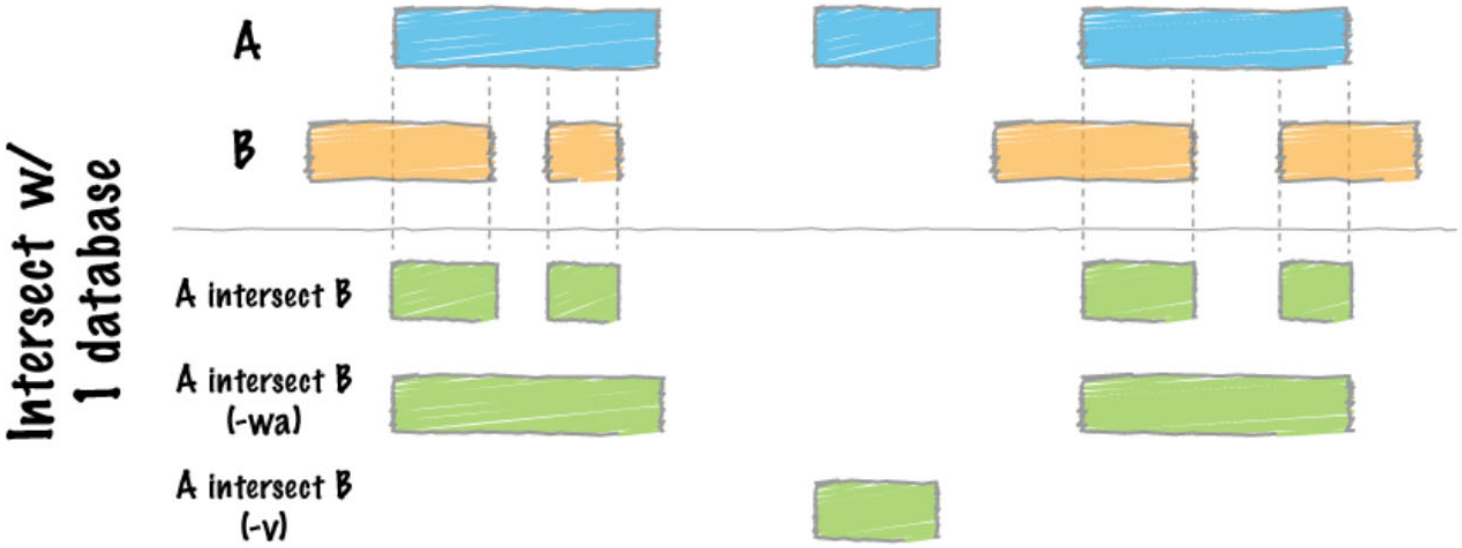
```
$ awk '{ total= $4 + $5 + $6; avg=total/3; print $0"\t"avg}' foo.txt
```

or

```
$ awk '{ total=0; for (i=4; i<=6; i++) total=total+$i; avg=total/3; print $0"\t"avg }' foo.txt
```

intersect from bedtools (intersectBed)

- Find overlaps between two sets of genomic features



https://bedtools.readthedocs.io/en/latest/content/tools/intersect.html

intersectBed: Examples

$ head -2 HumanGenesPlusMinus3kb.bed # showing file of gene coordinates

chr1	50899700	50905978	ENSG00000271782_RP5-850O15.4
chr1	103814769	103831355	ENSG00000232753_RP11-347K2.1

$ head -2 peaks.bed # showing file of genome regions (binding peaks)

chr1	19921	20016	MACS_peak_1	50
chr1	20025	20890	MACS_peak_2	568

To find: For each gene, is there an overlapping peak?

$ intersectBed -wa -wb -a HumanGenesPlusMinus3kb.bed -b peaks.bed | head -2

chr1	45956538	45968751	ENSG00000236624_CCDC163P	chr1	45955389	45956863	MACS_peak_3385	1192.24
chr1	45956538	45968751	ENSG00000236624_CCDC163P	chr1	45957202	45957380	MACS_peak_3386	121.87

To find: For each peak, does it overlap a gene?

$ intersectBed -wa -wb -a peaks.bed -b HumanGenesPlusMinus3kb.bed | head -2

chr1	19921	20016	MACS_peak_1	50.12	chr1	11363	32806	ENSG00000227232_WASH7P
chr1	20025	20890	MACS_peak_2	568.43	chr1	11363	32806	ENSG00000227232_WASH7P

Lots of other options: how much overlap? Is strand important? Print even if no match?

Summarize by Columns: groupBy (from bedtools)

Input file must be pre-sorted by grouping column(s)!

input

Ensembl Gene ID	Ensembl Transcript ID	Symbol
ENSG00000281518	ENST00000627423	FOXO6
ENSG00000281518	ENST00000630406	FOXO6
ENSG00000280680	ENST00000625523	HHAT
ENSG00000280680	ENST00000627903	HHAT
ENSG00000280680	ENST00000626327	HHAT
ENSG00000281614	ENST00000629761	INPP5D
ENSG00000281614	ENST00000630338	INPP5D

-g grpCols	column(s) for grouping
-c -opCols	column(s) to be summarized
-o	Operation(s) applied to opCol: sum, count, min, max, mean, median, stdev, collapse (comma-sep list) distinct (non-redundant comma-sep list)

Print the gene ID (1st column), the gene symbol , and a list of transcript IDs (2nd field)

$ sort -k1,1 Ensembl_info.txt | groupBy -g 1 -c 3,2 -o distinct,collapse

Partial output

Ensembl Gene ID	Symbol	Ensembl Transcript ID
ENSG00000281518	FOXO6	ENST00000627423,ENST00000630406
ENSG00000280680	HHAT	ENST00000625523,ENST00000626327,ENST00000627903

Shell Flavors

- Syntax (for scripting) depends the shell

  echo $SHELL # /bin/bash (on tak)

- bash is common and the default on tak.
- Some Linux shells (incomplete listing):

Shell	Name
sh	Bourne
bash	**Bourne-Again**
ksh	Korn shell
csh	C shell

Shell script advantages

- Automation: avoid having to retype the same commands many times
- Ease of use and more efficient
- Outline of a script:

#!/bin/bash ⟵ shebang: interprets how to run the script

commands… ⟵ *set of* commands used in the script

#comments ⟵ write comments using "#"

- Commonly used extension for script is .sh (eg. foo.sh), file must have executable permission

Bash Shell: ‘for’ loop

- Process multiple files with one command
- Reduce computational time with many cluster nodes

```
for mySam in `/bin/ls *.sam`
do
        bsub  wc -l $mySam
done
```

When referring to a variable, $ is needed before the variable name ($mySam), but $ is not needed when defining it (mySam).

Identical one-line command:

```
for samFile in `/bin/ls *.sam`; do bsub wc -l $samFile; done
```

Shell script example

```
#!/bin/bash
# Sample command: ./SAM_to_BAM_sort_index.sh *.sam

for filename in "$@" # <!-- loops over arguments
do
        printf "\nProcessing $filename ....\n"
        # strip extension (.sam)
        name=`basename $filename .sam`

        # Only continue if successful. (&&)
        samtools view -bS $filename > $name.bam &&
        samtools sort $name.bam -o $name.sorted.bam &&
        samtools index $name.sorted.bam &&
        rm $name.bam &&
        printf "Created and indexed $name.sorted.bam\n"
done

printf "\nAll done!\n\n"
```

 # You can use editors such as nedit, gedit, emacs, or pico to create shell scripts.
 # If you use Windows or Macs text editor, save as simple text format.
 # Special hidden Windows characters can be be removed with fromdos command

Accessing Shared Resources at Whitehead

- Linux
  - **/nfs/BaRC_Public/BaRC_code**
  - **/nfs/BaRC_training**
  - **/lab/solexa_public**
- Windows (access using *Start Menu → Search*)
  - **\\wi-files1\BaRC_Public \BaRC_code**
  - **\\wi-files1\BaRC_training**
  - **\\wi-bigdata\solexa_public**
- Macs (access using *Go → Connect to Server…*)
  - **smb://wi-files1/BaRC_Public/BaRC_code**
  - **smb://wi-files1/BaRC_training**
  - **smb://wi-bigdata/solexa_public**

Where's my lab's share?

- http://it.wi.mit.edu/systems/file-storage/lab-share-paths

Best practices

- Save all good (and perhaps bad) commands
- Add comments about what you're doing, and why
- Check what your think each command does
- Plan to make your records understandable years in the future
- Be aware of the best Linux permissions for each file
- Treat your time as valuable, and use commands to save your valuable time
- Share what you learn and create!

Further Reading

- BaRC one liners:
  - http://bioinfo.wi.mit.edu/bio/bioinfo/scripts/#unix
- Linux Info for Bioinfo:
  - http://bioinfo.wi.mit.edu/bio/education/unix_intro.php
- Bash Guide for Beginners:
  - http://tldp.org/LDP/Bash-Beginners-Guide/html/

Upcoming Hot Topics

http://barc.wi.mit.edu/education/hot_topics/upcoming/

- An introduction to R - Thursday, Nov 3rd, 1 - 2:30pm
- An introduction to R graphics - Thursday Nov 17th, 1 - 2:30pm

- Programming with python (with Software Carpentry staff) - Thursday, Dec 8th, 9am - 5pm
- Version control with git (with Software Carpentry staff) - Friday Dec 9th, 1 - 5pm

- Analytical project management [by request]