Linux for Biologists – Part 3

Robert Bukowski
Institute of Biotechnology
Bioinformatics Facility
(aka Computational Biology Service Unit - **CBSU**)

http://cbsu.tc.cornell.edu/lab/doc/Linux_workshop_Part3.pdf

Running applications

Running applications

- ❑ Very much like running system commands
- ❑ (Very) general syntax

```
<path_to_application_executable> <options>
```

- ❑ A few quick examples:

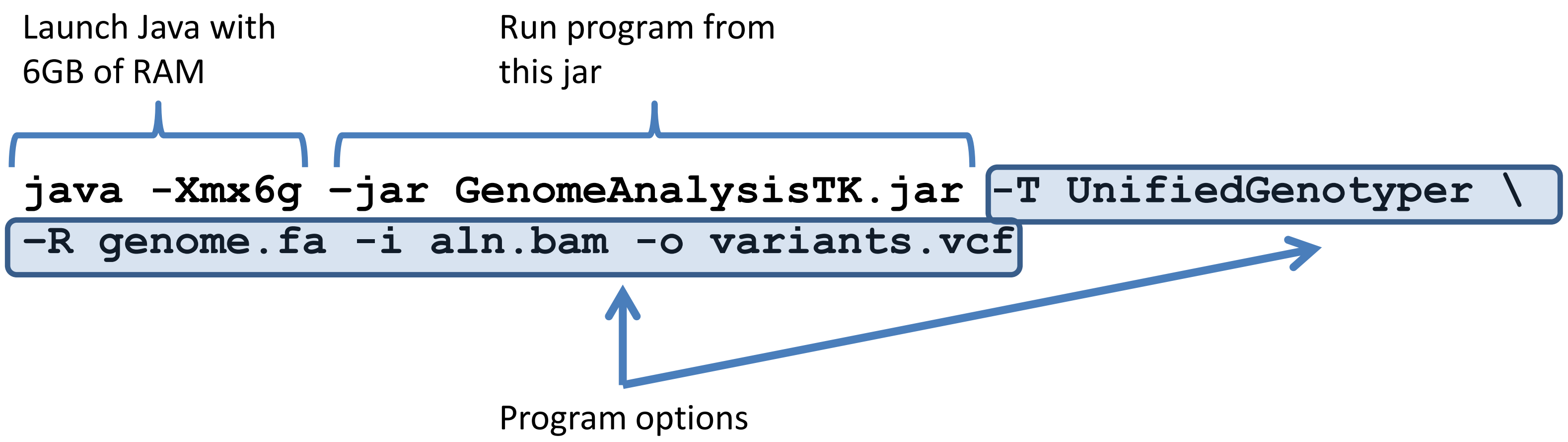
```
blastall  -p blastx -b 1 -d ./databases/swissprot -i seq_tst.fa

samtools  flagstat alignments.bam

tophat  -p 7 -o B_L1-1 --transcriptome-index  ZmB73_5a_WGS \
--no-novel-juncs  genome/maize reads_R1.fastq.gz reads_R2.fastq.gz
```

Running applications

- Why can we call, say, **samtools** by just typing **`samtools`** rather than the full path (in this case, **`/programs/bin/samtools/samtools)`** ?
  - Because of the search path environment variable which is defined for everybody. When you type **`samtools`**, the system tries each directory on the search path one by one until it finds the corresponding executable.
  - `which samtools` *(tells us where on disk the command* bwa *is located)*
  - `echo $PATH` *(displays the search path)*
  - **Note**: the current directory **./** is **NOT** in the search path. If you need to run a program located, say in your home directory, you need to precede it with **./**, for example, **`./my_program`**
  - **Note:** majority of executables **are NOT in search path** – they need to be launched using **full path**.
    - Visit https://cbsu.tc.cornell.edu/lab/labsoftware.aspx to find out the path to your application

Running applications

- How to run **Java** applications?
- Java programs usually come packaged in so-called **jars**
- Java program is launched by running the **java virtual machine** with the appropriate **jar** as an argument
- Example:



```
java -Xmx6g -jar GenomeAnalysisTK.jar -T UnifiedGenotyper \
-R genome.fa -i aln.bam -o variants.vcf
```

Running applications

- Need to know what program(s) are relevant for your particular problem

- Need to know what a given program does and how to use it

    - Visit our software page http://cbsu.tc.cornell.edu/lab/labsoftware.aspx

        - Links to manuals (all options explained, examples given, test data available)

        - Specific hints on running in BioHPC Lab environment

- Getting quick help – run **command without any options**, or sometimes with **`–h`** or **`--help`**

    - Should print a list of options with very short descriptions

Running applications example: BLAST

- Input:
  - **FASTA file** with query sequences
    - We will use 9 random human cDNA sequences
  - **Database** of known sequences with which the query is to be compared
    - We will use **Swissprot** set of amino acid sequences
      - Need to translate each cDNA query in 6 frames and align to Swissprot templates
- Output
  - Text file describing hits
- Program to run: **`blastall`**

Running applications example: BLAST

prepare input

- ❑ Create your local scratch directory (if not yet done) and a sub-directory **`blast_test`** where this exercise will be run

```
mkdir /workdir/bukowski
cd /workdir/bukowski
mkdir blast_test
cd blast_test
```

- ❑ Copy file with query sequences to the exercise directory:

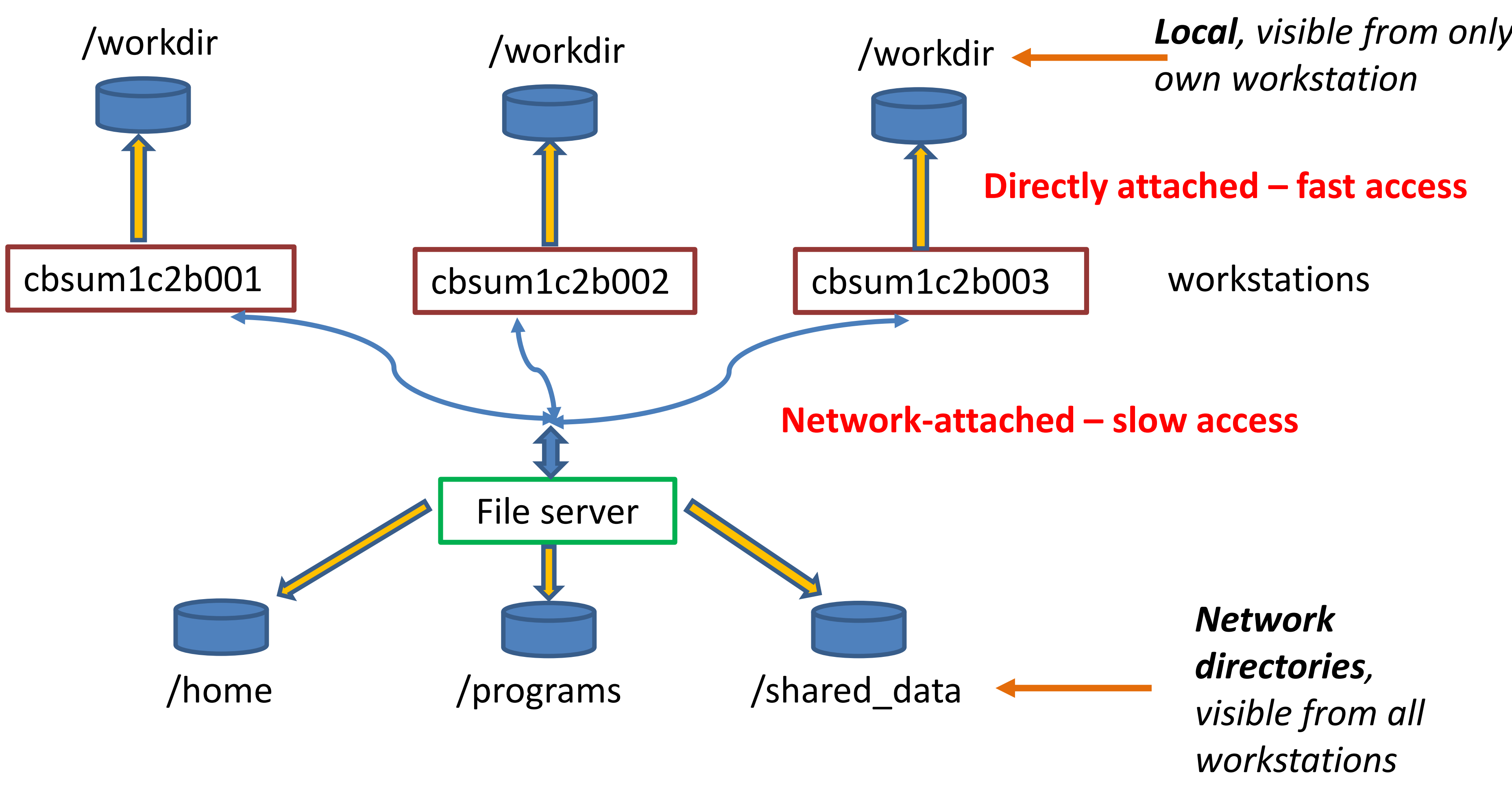
```
cp /shared_data/Linux_workshop/seq_tst.fa .
```

- ❑ Copy Swissprot BLAST database (we'll make a separate directory for it)

```
mkdir databases
cp /shared_data/Linux_workshop/databases/swissprot* ./databases
```

- ❑ Verify that the files have been copied (use **`ls`** command)

Reminder: local vs. network directories in BioHPC Lab


/workdir
/workdir
/workdir
***Local**, visible from only own workstation*
Directly attached – fast access
cbsum1c2b001
cbsum1c2b002
cbsum1c2b003
workstations
Network-attached – slow access
File server
/home
/programs
/shared_data
***Network directories**, visible from all workstations*

Files frequently read and/or written (like input and output from an application being run) must be located on **local directories** (on BioHPC Lab machines: **/workdir**)

Running applications example: BLAST

run the program

- <u>Very</u> general syntax for launching applications:

```
<path_to_application_executable>  [options] >& log
```

- **In our specific case:**

$$\underbrace{\texttt{blastall}}_{\text{Path to application executable}}\ \underbrace{\texttt{-p blastx -b 1 -d ./databases/swissprot -i seq\_tst.fa}}_{\text{Program options}}\ \underbrace{\texttt{>\& run.log}}_{\text{Screen output redirect}}$$

- Options used:
  - -p: type of search (**blastx**: compare 6-frame translations of DNA to AA sequences)
  - -b: number of database sequences to show alignments for
  - -d: path to database files
  - -i: input file (with query sequences in fasta format)
- For full set of options, run

```
blastall  | more
```

Running applications example: BLAST

running the program

```
blastall -p blastx -b 1 -d ./databases/swissprot -i seq_tst.fa >& run.log
```

- The program will run for about 1 minute and then write the output to the file **`run.log`** (STDOUT and STDERR streams combined)
  - Often output will appear in **`run.log`** gradually as a program is running

- For larger queries, the run will take (much) longer and produce more output…
  - 10,000 similar query sequences run using a similar command would take about 24 hours

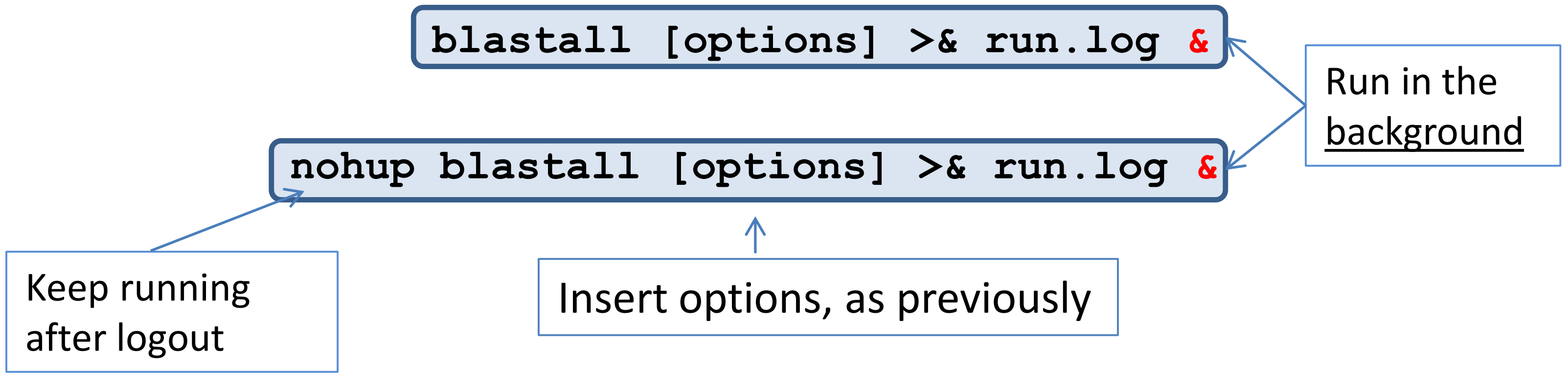
Running a program, cnt.

- Running a program in the background

  - Normally, the program will run to completion (or crash), blocking the terminal window

  - By putting an “&” at the end of command, we can send the program to the **background**

    - Terminal prompt will return immediately – you will be able to continue working
    - Good for long-running programs (most programs of interest…)
    - Can run multiple programs simultaneously if more then 1 processor available on a machine (more about it later)
    - If all screen output redirected to disk, you may **log out** and leave the program running (to make sure, use **nohup** before the command)



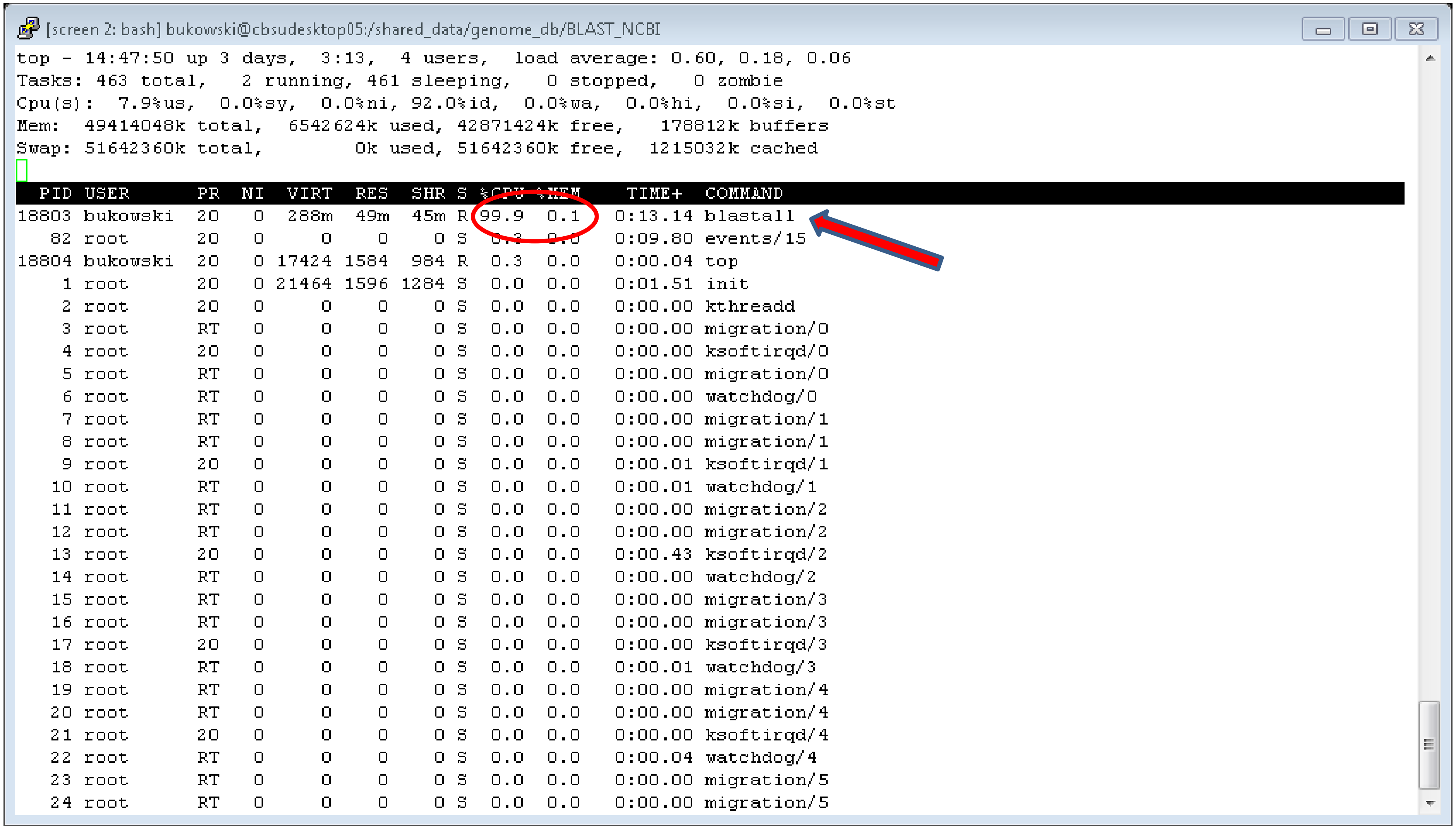
```
blastall [options] >& run.log &
```

```
nohup blastall [options] >& run.log &
```

Running applications

<u>Checking on your application: the **`top`** command</u>

To exit – just type **q**



[screen 2: bash] bukowski@cbsudesktop05:/shared_data/genome_db/BLAST_NCBI

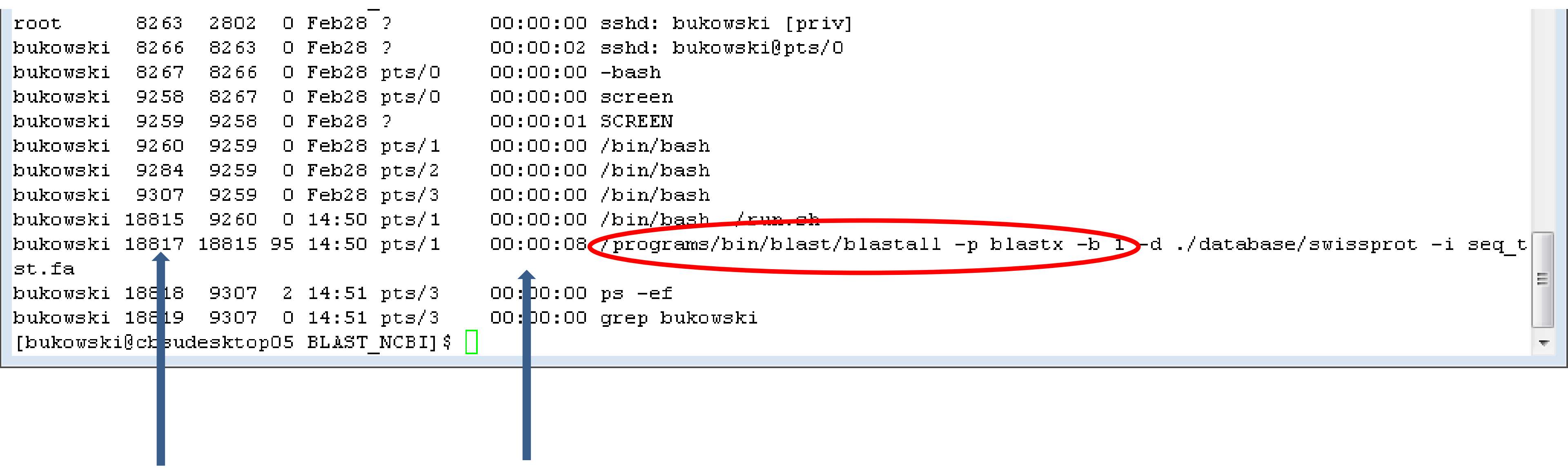
```
top - 14:47:50 up 3 days,  3:13,  4 users,  load average: 0.60, 0.18, 0.06
Tasks: 463 total,   2 running, 461 sleeping,   0 stopped,   0 zombie
Cpu(s):  7.9%us,  0.0%sy,  0.0%ni, 92.0%id,  0.0%wa,  0.0%hi,  0.0%si,  0.0%st
Mem:  49414048k total,  6542624k used, 42871424k free,   178812k buffers
Swap: 51642360k total,        0k used, 51642360k free,  1215032k cached

  PID USER      PR  NI  VIRT  RES  SHR S %CPU %MEM    TIME+  COMMAND
18803 bukowski  20   0  288m  49m  45m R 99.9  0.1   0:13.14 blastall
   82 root      20   0     0    0    0 S  0.3  0.0   0:09.80 events/15
18804 bukowski  20   0 17424 1584  984 R  0.3  0.0   0:00.04 top
    1 root      20   0 21464 1596 1284 S  0.0  0.0   0:01.51 init
    2 root      20   0     0    0    0 S  0.0  0.0   0:00.00 kthreadd
    3 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/0
    4 root      20   0     0    0    0 S  0.0  0.0   0:00.00 ksoftirqd/0
    5 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/0
    6 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 watchdog/0
    7 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/1
    8 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/1
    9 root      20   0     0    0    0 S  0.0  0.0   0:00.01 ksoftirqd/1
   10 root      RT   0     0    0    0 S  0.0  0.0   0:00.01 watchdog/1
   11 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/2
   12 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/2
   13 root      20   0     0    0    0 S  0.0  0.0   0:00.43 ksoftirqd/2
   14 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 watchdog/2
   15 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/3
   16 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/3
   17 root      20   0     0    0    0 S  0.0  0.0   0:00.00 ksoftirqd/3
   18 root      RT   0     0    0    0 S  0.0  0.0   0:00.01 watchdog/3
   19 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/4
   20 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/4
   21 root      20   0     0    0    0 S  0.0  0.0   0:00.00 ksoftirqd/4
   22 root      RT   0     0    0    0 S  0.0  0.0   0:00.04 watchdog/4
   23 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/5
   24 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/5
```

Running applications, cnt.

<u>Checking on your application:</u>

the **`ps`** command – display info about all your processes – one of them should be **`blastall`**

```
ps -ef | grep bukowski
```



```
root       8263  2802  0 Feb28 ?        00:00:00 sshd: bukowski [priv]
bukowski   8266  8263  0 Feb28 ?        00:00:02 sshd: bukowski@pts/0
bukowski   8267  8266  0 Feb28 pts/0    00:00:00 -bash
bukowski   9258  8267  0 Feb28 pts/0    00:00:00 screen
bukowski   9259  9258  0 Feb28 ?        00:00:01 SCREEN
bukowski   9260  9259  0 Feb28 pts/1    00:00:00 /bin/bash
bukowski   9284  9259  0 Feb28 pts/2    00:00:00 /bin/bash
bukowski   9307  9259  0 Feb28 pts/3    00:00:00 /bin/bash
bukowski  18815  9260  0 14:50 pts/1    00:00:00 /bin/bash ./run.sh
bukowski  18817 18815 95 14:50 pts/1    00:00:08 /programs/bin/blast/blastall -p blastx -b 1 -d ./database/swissprot -i seq_t
st.fa
bukowski  18818  9307  2 14:51 pts/3    00:00:00 ps -ef
bukowski  18819  9307  0 14:51 pts/3    00:00:00 grep bukowski
[bukowski@cbsudesktop05 BLAST_NCBI]$ 
```

Try **`man ps`** for more info about the **`ps`** command.

Running applications

❑ Stopping applications

- If the application is running in the foreground (i.e., without “&”), it can be stopped with **Ctrl-C** (press and hold the Ctrl key, then press the “C” key) issued from the window (terminal) it is running in.

- If the application is running in the **background** (i.e., with “**&**”), it can be stopped with the **`kill`** command

```
kill -9 <PID>
```

Where <PID> is the process id obtained rom the **ps** command. For example, to terminate the **blastall** process form the previous slide, we would use

```
kill -9 18817
```

Try **`man kill`** for more info about the **`kill`** command.

Keeping a program running in the background after you log out or disconnect

Option 1: Use **nohup** (as on previous slide). Of course, you can use this also with options 1 and 2.

Option 2: Start a program in a terminal within a **VNC session**

- the session keeps running after VNC connection is killed
- you can reconnect to VNC session later

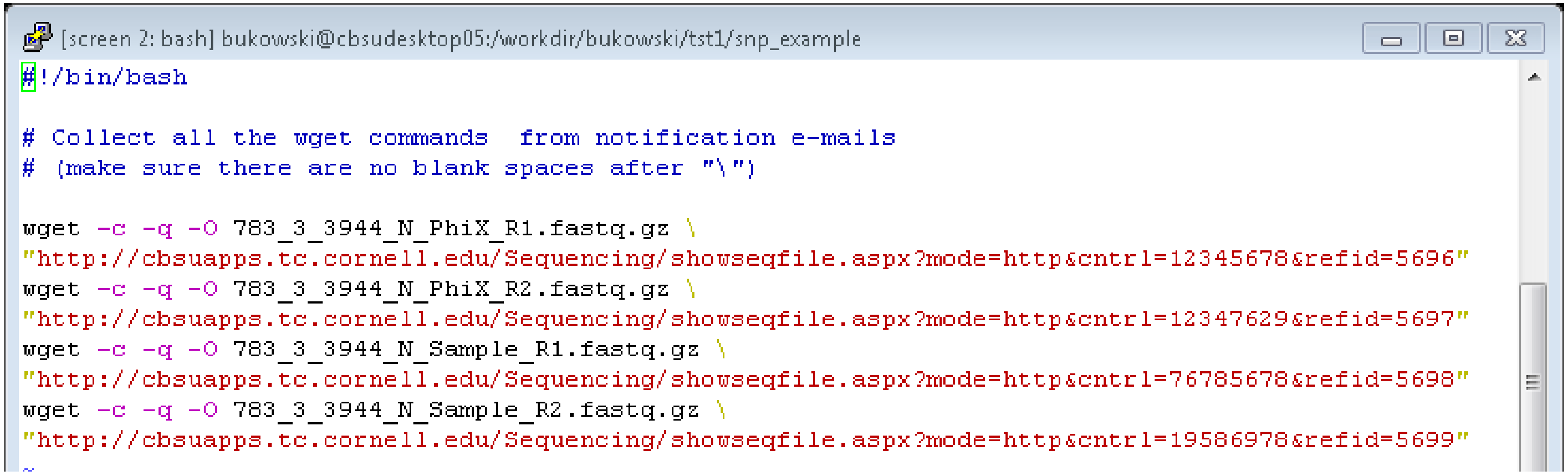
Option 3: Start a program within a **screen** window

- all such windows keep running after you disconnect using "Ctrl-a d" or by killing terminal window
- you can reconnect to the whole session later

Shell scripting

Example we already talked about: Downloading Illumina sequencing results

Script **`download.sh`** is sent as attachment to notification e-mail from the sequencing facility



[screen 2: bash] bukowski@cbsudesktop05:/workdir/bukowski/tst1/snp_example

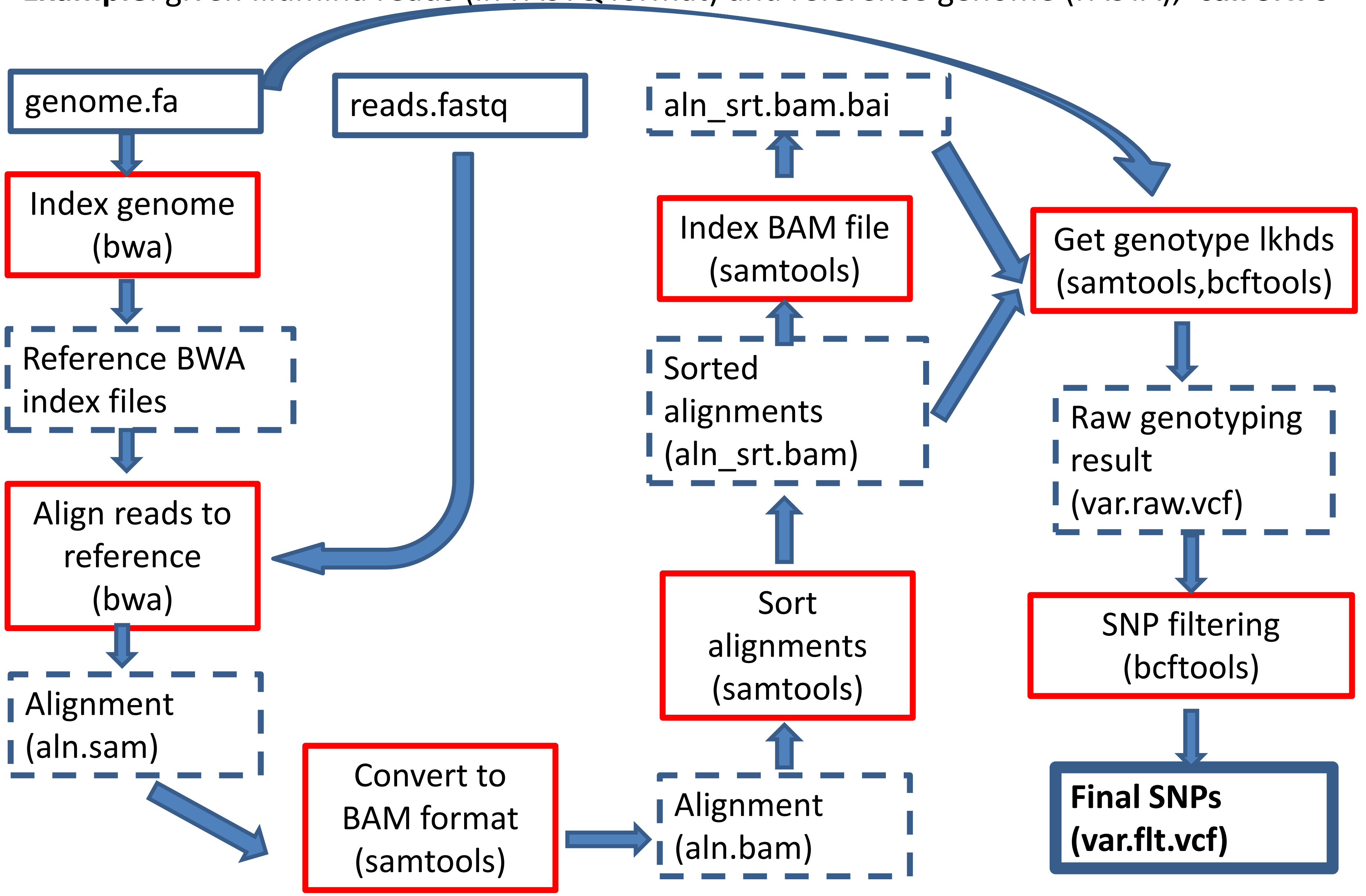
```
#!/bin/bash

# Collect all the wget commands  from notification e-mails
#  (make sure there are no blank spaces after "\")

wget -c -q -O 783_3_3944_N_PhiX_R1.fastq.gz \
"http://cbsuapps.tc.cornell.edu/Sequencing/showseqfile.aspx?mode=http&cntrl=12345678&refid=5696"
wget -c -q -O 783_3_3944_N_PhiX_R2.fastq.gz \
"http://cbsuapps.tc.cornell.edu/Sequencing/showseqfile.aspx?mode=http&cntrl=12347629&refid=5697"
wget -c -q -O 783_3_3944_N_Sample_R1.fastq.gz \
"http://cbsuapps.tc.cornell.edu/Sequencing/showseqfile.aspx?mode=http&cntrl=76785678&refid=5698"
wget -c -q -O 783_3_3944_N_Sample_R2.fastq.gz \
"http://cbsuapps.tc.cornell.edu/Sequencing/showseqfile.aspx?mode=http&cntrl=19586978&refid=5699"
```

Copy **`download.sh`** to your Linux machine and run as a script

```
sh ./download.sh
```

Script for a complex task: SNP-calling

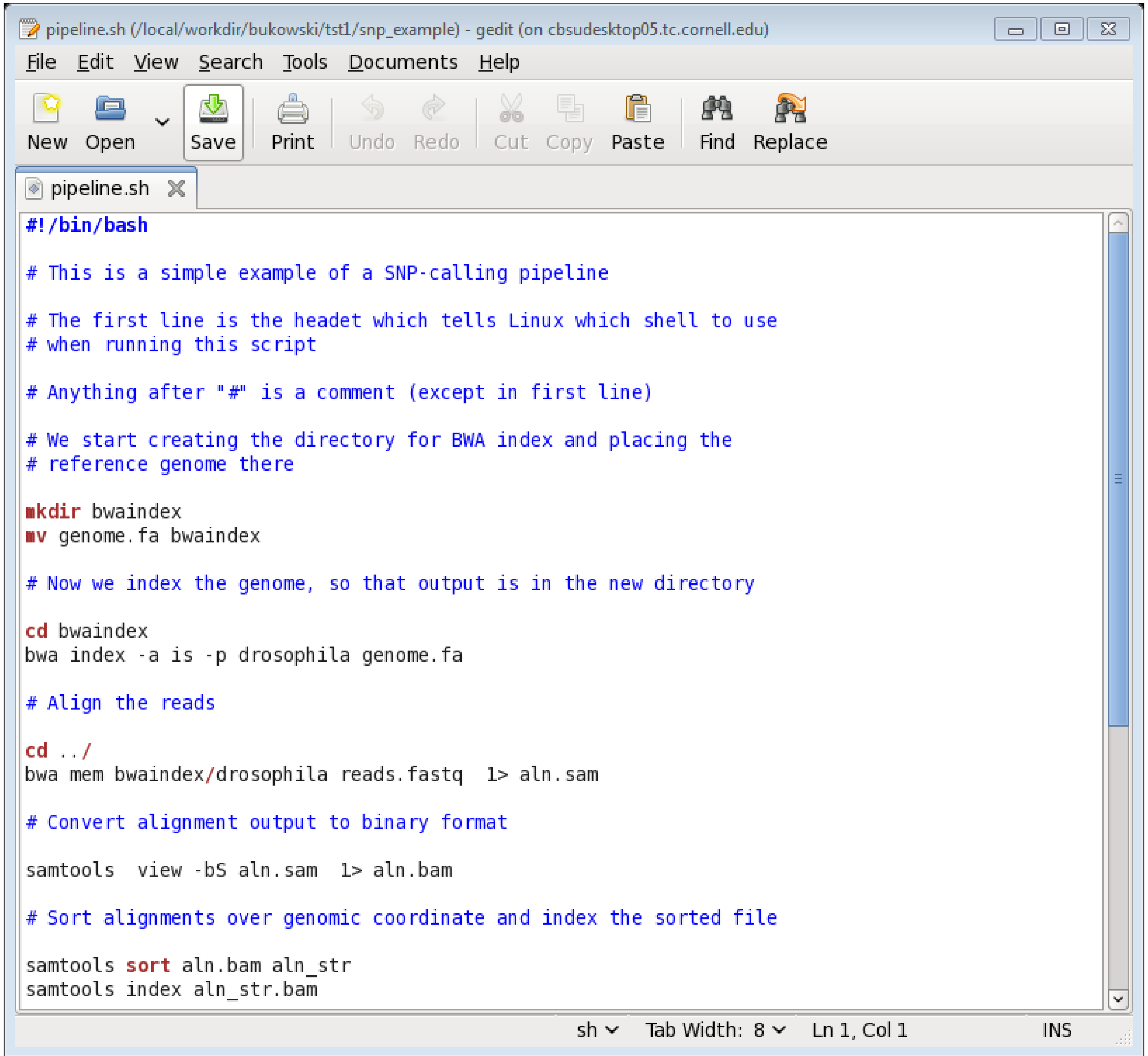
Example: given Illumina reads (in FASTQ format) and reference genome (FASTA), **call SNPs**


genome.fa
reads.fastq
aln_srt.bam.bai
Index genome (bwa)
Index BAM file (samtools)
Get genotype lkhds (samtools,bcftools)
Reference BWA index files
Sorted alignments (aln_srt.bam)
Raw genotyping result (var.raw.vcf)
Align reads to reference (bwa)
Sort alignments (samtools)
SNP filtering (bcftools)
Alignment (aln.sam)
Convert to BAM format (samtools)
Alignment (aln.bam)
Final SNPs (var.flt.vcf)

Scripts: tools for executing complex tasks

- Sequence of steps on previous slide is an example of a **pipeline**
  - Each step corresponds to (typically) one instance of a program or command
  - Input files used in a step are (typically) generated in preceding steps
  - Some steps may run quite long (depends on amount of input data and size of reference)
  - Executing each step in a terminal as a command is possible, but tedious and hard to repeat (for example, with a new input data)
  - Remedy: write a **shell script** – <u>a text file with commands</u>

Shell script: a set of commands (and comments) in a text file


pipeline.sh (/local/workdir/bukowski/tst1/snp_example) - gedit (on cbsudesktop05.tc.cornell.edu)
File Edit View Search Tools Documents Help
New Open Save Print Undo Redo Cut Copy Paste Find Replace
pipeline.sh
sh Tab Width: 8 Ln 1, Col 1 INS

```
#!/bin/bash

# This is a simple example of a SNP-calling pipeline

# The first line is the headet which tells Linux which shell to use
# when running this script

# Anything after "#" is a comment (except in first line)

# We start creating the directory for BWA index and placing the
# reference genome there

mkdir bwaindex
mv genome.fa bwaindex

# Now we index the genome, so that output is in the new directory

cd bwaindex
bwa index -a is -p drosophila genome.fa

# Align the reads

cd ../
bwa mem bwaindex/drosophila reads.fastq  1> aln.sam

# Convert alignment output to binary format

samtools  view -bS aln.sam  1> aln.bam

# Sort alignments over genomic coordinate and index the sorted file

samtools sort aln.bam aln_str
samtools index aln_str.bam
```

This is a fragment of an actual script implementing the SNP-calling pipeline.

Run the whole script as homework – see the end of this presentation

Shell scripts

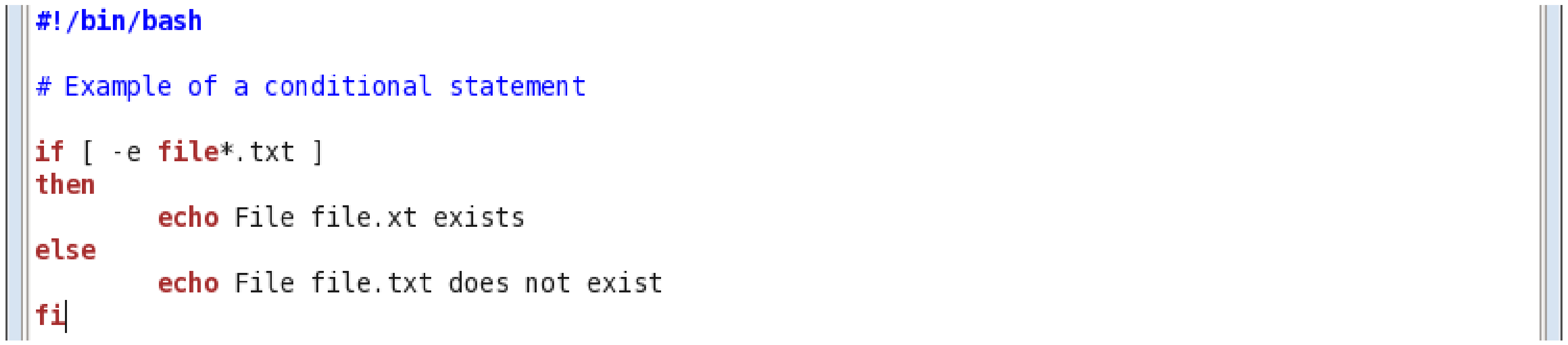
- First line should be **`#!/bin/bash`** (indicates the shell used to interpret the script)
  - If absent, default shell will be used (bash)
- Everything in a line following "**#**" is a **comment**
- May include system commands (like **`cp, mv, mkdir`**, …) and commands launching programs (**`blastall, bwa, samtools`**, …)
- Commands will be executed "in the order of appearance"
- Long lines can be broken with "\" character
  - The "\" character must be the last one in a line (no blank spaces after it)
- Script (e.g., **`my_script.sh`**, in the current directory) can be run as in the following:

```
bash ./my_script.sh >& my_script.log &
./my_script.sh >& my_script.log  &
```

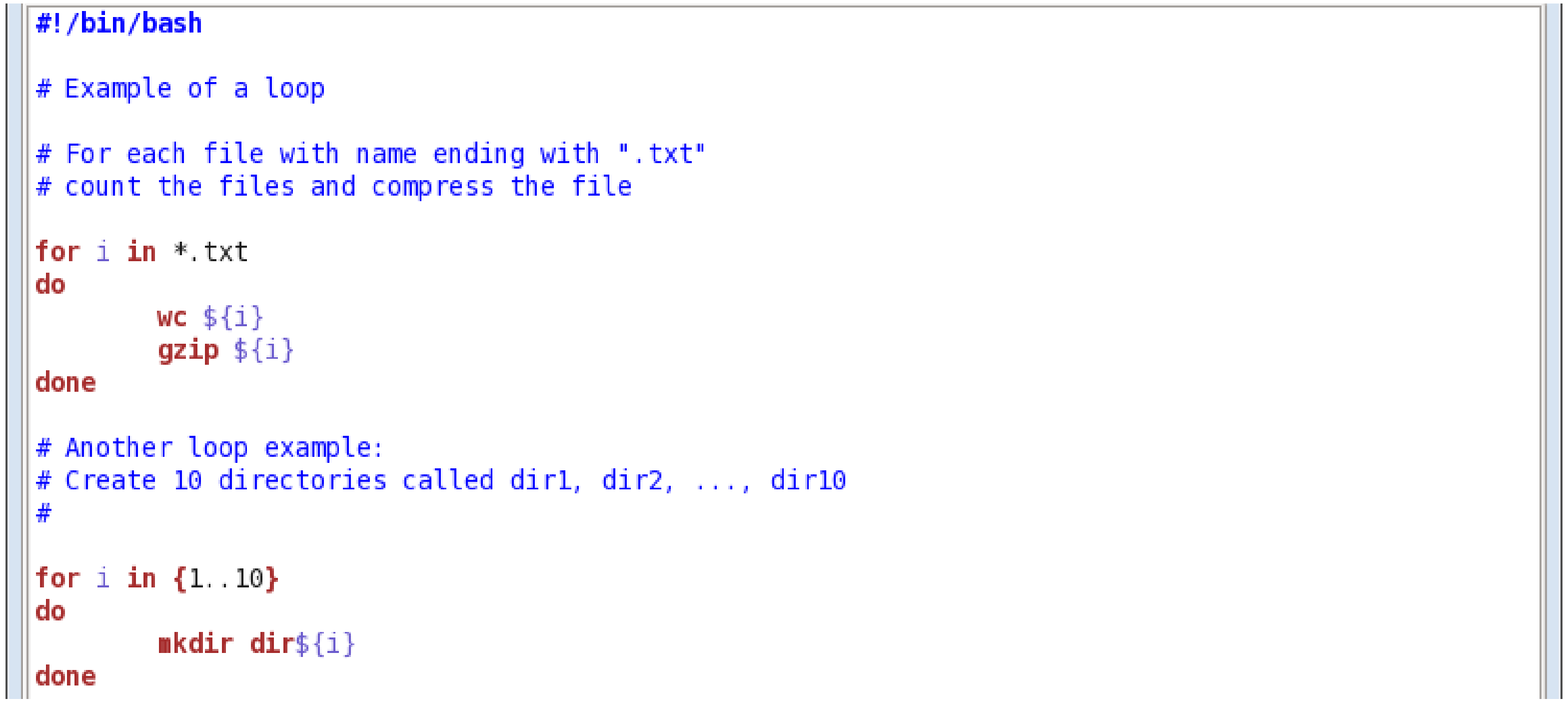
- The second command will work if the file **`my_script.sh`** is <u>made executable </u>with the command

```
chmod u+x my_script.sh
```

Shell scripts: conditionals and loops



```
#!/bin/bash

# Example of a conditional statement

if [ -e file*.txt ]
then
        echo File file.xt exists
else
        echo File file.txt does not exist
fi
```



```
#!/bin/bash

# Example of a loop

# For each file with name ending with ".txt"
# count the files and compress the file

for i in *.txt
do
        wc ${i}
        gzip ${i}
done

# Another loop example:
# Create 10 directories called dir1, dir2, ..., dir10
#

for i in {1..10}
do
        mkdir dir${i}
done
```

Exercise

(see end of slide deck)

simple SNP-calling pipeline

Objective: align (simulated) Illumina reads to D. Melanogaster genome using **BWA** aligner and call variants using **samtools**

More about scripting

Multiple scripting tools available

- **shell** (bash, tcsh – good for stitching together shell commands)
- **perl** (very popular in biology, due to BioPerl module package)
- **python** (good numerical analysis tools – NumPy, SciPy packages)
- **awk** (mostly text parsing and processing)
- **sed** (mostly text parsing and processing)
- **R** (rich library of numerical analysis and statistical functions)

Using multiple processors

Recommended reading:
Efficient use of CPUs/cores on BioHPC Lab machines
http://cbsu.tc.cornell.edu/lab/doc/using_BioHPC_CPUs.pdf

Multiple processors

Using **BLAST** to search **swissprot** database for matches of 10,000 randomly chosen human cDNA sequences (swissprot is a good example of a small memory footprint).

machine	CPU available	cores available	cores used	time (hrs)	speedup (in machine)
cbsulm10	4	64	**64**	0.931	27.506
cbsulm10	4	64	**16**	1.962	13.056
cbsulm10	4	64	**1**	25.619	1.000
cbsumm15	2	24	**24**	2.058	12.117
cbsumm15	2	24	**12**	2.593	9.616
cbsumm15	2	24	**1**	24.930	1.000
cbsum1c2b008	2	8	**8**	4.193	6.717
cbsum1c2b008	2	8	**1**	28.161	1.000

Using **BLAST** to search **nr** database for matches of 2,000 randomly chosen human cDNA sequences (nr is a good example of a large memory footprint).

machine	CPU available	cores available	cores used	time (hrs)	speedup (in machine)
cbsulm10	4	64	**64**	10.97	2.222
cbsulm10	4	64	**16**	24.37	1.000
cbsumm15	2	24	**24**	26.10	2.140
cbsumm15	2	24	**12**	55.85	1.000

Multiple processors

- It is VERY important to use multiple cores. BLAST on 64 cores takes only 0.931 hours (2K cDNA vs swissprot), the same run on a single core takes over 25 hours!

- **Speedup** is not directly proportional to the number of cores. Most often it is less than expected, but still sufficiently large to justify the effort. 64 cores compared to 1 core in swissprot example give 27.5 speedup rate, much less than 64-fold, but still large!

- Speedup depends on the machine (hardware), program (algorithm), and parameters (e.g., nr vs swissport). When using **nr** database on cbsumm15 the speedup between 12 and 24 cores is 2.14. For **swissprot** on the same machine it is only 1.26.

  - It is often a good idea to run a short example first (if possible) on a subset of data to figure out the optimal number of cores.

Multiple processors

Three ways to utilize multiple CPU cores on a machine:

- ❑ Using a given program’s built-in parallelization
- ❑ Simultaneously executing several programs in the background
- ❑ Using a “driver” program to execute multiple tasks in parallel

Multiple processors

- Take advantage of a program's built-in parallelism invoked with an option
  - read documentation to find out if your program has this feature
  - Look for keywords like "multithreading", "parallel execution", "multiple processors", etc.

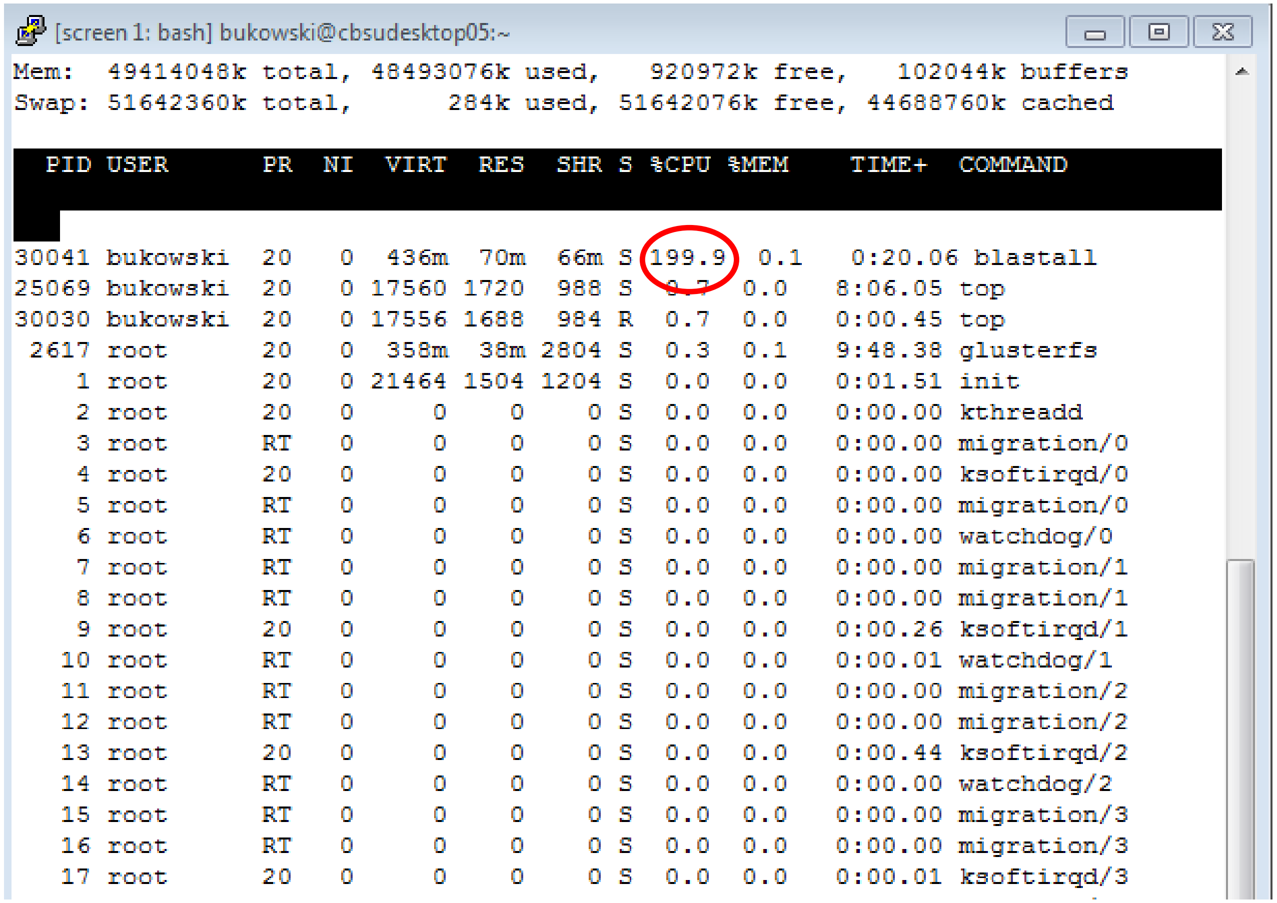
A few examples:

```
blastall -a 8 [other options]

blast+ -num_threads 8 [other options]

tophat -p 8 [other options]

cuffdiff -p 8 [other options]

bwa -t 8 [other options]

bowtie -p 8 [other options]
```

Remember speedup is not perfect, so optimal number of threads needs to be optimized by trial and error using subset of input data

Multiple processors

```
blastall -a 2 -p blastx -b 1 -d ./databases/swissprot -i seq_tst.fa
```



[screen 1: bash] bukowski@cbsudesktop05:~

```
Mem:  49414048k total, 48493076k used,   920972k free,   102044k buffers
Swap: 51642360k total,       284k used, 51642076k free, 44688760k cached

  PID USER      PR  NI  VIRT  RES  SHR S %CPU %MEM    TIME+  COMMAND

30041 bukowski  20   0  436m  70m  66m S 199.9  0.1   0:20.06 blastall
25069 bukowski  20   0 17560 1720  988 S  0.7  0.0   8:06.05 top
30030 bukowski  20   0 17556 1688  984 R  0.7  0.0   0:00.45 top
 2617 root      20   0  358m  38m 2804 S  0.3  0.1   9:48.38 glusterfs
    1 root      20   0 21464 1504 1204 S  0.0  0.0   0:01.51 init
    2 root      20   0     0    0    0 S  0.0  0.0   0:00.00 kthreadd
    3 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/0
    4 root      20   0     0    0    0 S  0.0  0.0   0:00.00 ksoftirqd/0
    5 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/0
    6 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 watchdog/0
    7 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/1
    8 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/1
    9 root      20   0     0    0    0 S  0.0  0.0   0:00.26 ksoftirqd/1
   10 root      RT   0     0    0    0 S  0.0  0.0   0:00.01 watchdog/1
   11 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/2
   12 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/2
   13 root      20   0     0    0    0 S  0.0  0.0   0:00.44 ksoftirqd/2
   14 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 watchdog/2
   15 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/3
   16 root      RT   0     0    0    0 S  0.0  0.0   0:00.00 migration/3
   17 root      20   0     0    0    0 S  0.0  0.0   0:00.01 ksoftirqd/3
```

- >100% CPU indicates the program is **multithreaded**
  - Multiple threads within a single process rather than multiple processes

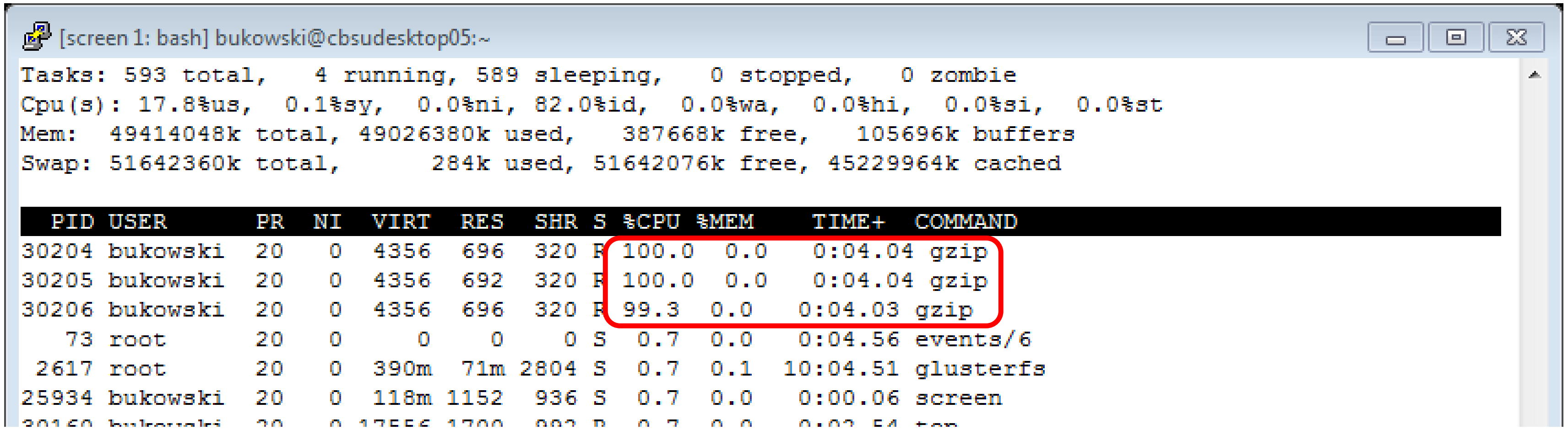
Multiple processors

- Simultaneously executing several programs in the background

Example: suppose we have to compress (gzip) several files. We can simply launch multiple **gzip** commands in the background, without waiting for previous ones to finish:

```
gzip file1 &
gzip file2 &
gzip file3 &
```

Multiple processes
(1 thread in each)



[screen 1: bash] bukowski@cbsudesktop05:~

```
Tasks: 593 total,   4 running, 589 sleeping,   0 stopped,   0 zombie
Cpu(s): 17.8%us,  0.1%sy,  0.0%ni, 82.0%id,  0.0%wa,  0.0%hi,  0.0%si,  0.0%st
Mem:  49414048k total, 49026380k used,   387668k free,   105696k buffers
Swap: 51642360k total,      284k used, 51642076k free, 45229964k cached

  PID USER      PR  NI  VIRT  RES  SHR S %CPU %MEM    TIME+  COMMAND
30204 bukowski  20   0  4356  696  320 R 100.0  0.0   0:04.04 gzip
30205 bukowski  20   0  4356  692  320 R 100.0  0.0   0:04.04 gzip
30206 bukowski  20   0  4356  696  320 R 99.3  0.0   0:04.03 gzip
   73 root      20   0     0    0    0 S  0.7  0.0   0:04.56 events/6
 2617 root      20   0  390m  71m 2804 S  0.7  0.1  10:04.51 glusterfs
25934 bukowski  20   0  118m 1152  936 S  0.7  0.0   0:00.06 screen
```

Multiple processors

What if in the previous example, we had, say, **3000** files instead of just 3, but **still only a few processors**?

Submitting all 3000 commands simultaneously in the background (in principle, it could be done painlessly using a script) would not work too well, because:

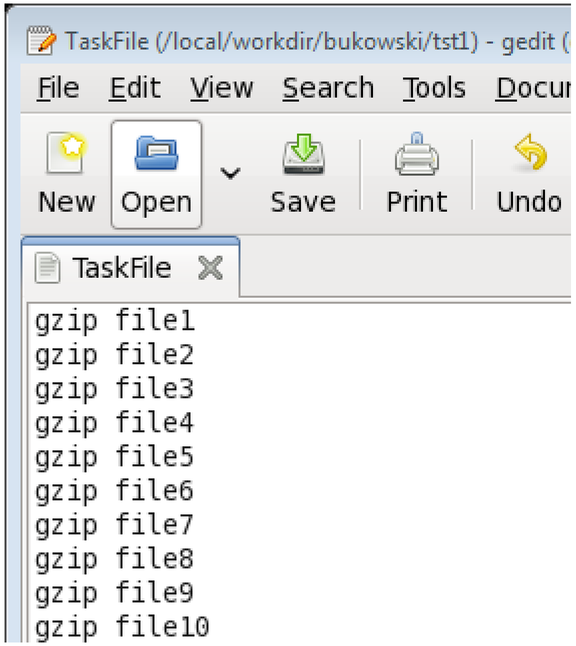
- ❑ Each processor would have to switch between many processes – possible, but inefficient
- ❑ With large number (and/or size) of files being processed, access to disk would become a bottleneck (i.e., processes would spend most of their time competing for access to disk)
  - ❑ Disk access (referred to as I/O – input/output) is always an issue for programs which do a lot of reading/writing (like **`gzip`**)
- ❑ As a result, we would get no speedup, or (more likely) **processing of all files in parallel would take longer than processing them one by one**

In situations like this (many short tasks and a few processors), we need a special “driver” tool to efficiently distribute the tasks.

Multiple processors

- ❑ Using a "driver" program to execute multiple tasks in parallel

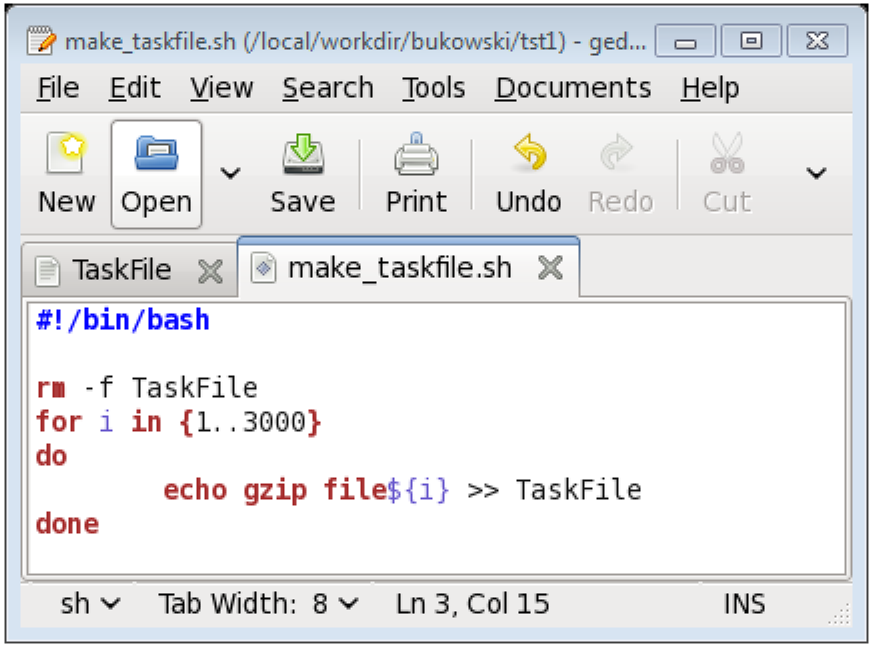
Example: create a file called (for example) **`TaskFile`**
(This is **NOT** a script, although it could be executed as such…)


TaskFile (/local/workdir/bukowski/tst1) - gedit (
File Edit View Search Tools Docu
New Open Save Print Undo
TaskFile

```
gzip file1
gzip file2
gzip file3
gzip file4
gzip file5
gzip file6
gzip file7
gzip file8
gzip file9
gzip file10
```

….. (up to **`file3000`**)

This long file can be created, for example, using the following shell script:


make_taskfile.sh (/local/workdir/bukowski/tst1) - ged...
File Edit View Search Tools Documents Help
New Open Save Print Undo Redo Cut
TaskFile
make_taskfile.sh
sh
Tab Width: 8
Ln 3, Col 15
INS

```
#!/bin/bash

rm -f TaskFile
for i in {1..3000}
do
        echo gzip file${i} >> TaskFile
done
```

Multiple processors

Then run the command (assuming the **`TaskFile`** and all **`file*`** files are in the current dir)

```
/programs/bin/perlscripts/perl_fork_univ.pl TaskFile NP >& log &
```

where **`NP`** is the number of processors to use (e.g., 10)

- **`perl_fork_univ.pl`** is an CBSU in-house "driver" script (written in perl)

- It will execute tasks listed in **`TaskFile`** using up to **`NP`** processors
  - The first **`NP`** tasks will be launched simultaneously
  - The **`(NP+1)`** th task will be launched right after one of the initial ones completes and a "slot" becomes available
  - The **`(NP+2)`** nd task will be launched right after another slot becomes available
  - …… etc., until all tasks are distributed

- Only up to **`NP`** tasks are running at a time (less at the end)

- All **`NP`** processors always kept busy (except near the end of task list) – **Load Balancing**

Mixed parallelization: running several simultaneous multi-threaded tasks (each processing different data) on a large machine (here: 64-core)

```
tophat -p 7 -o B_L1-1 --transcriptome-index genome/transcriptome/ZmB73_5a_WGS \
   --no-novel-juncs genome/maize \
   fastq/2284_6063_7073_C3AR7ACXX_B_L1-1_ATCACG_R1.fastq.gz \
   fastq/2284_6063_7073_C3AR7ACXX_B_L1-1_ATCACG_R2.fastq.gz >& B_L1-1.log &
tophat -p 7 -o B_L1-2 --transcriptome-index genome/transcriptome/ZmB73_5a_WGS \
   --no-novel-juncs genome/maize \
   fastq/2284_6063_7076_C3AR7ACXX_B_L1-2_TGACCA_R1.fastq.gz  \
   fastq/2284_6063_7076_C3AR7ACXX_B_L1-2_TGACCA_R2.fastq.gz >& B_L1-2.log &
tophat -p 7 -o B_L1-3 --transcriptome-index genome/transcriptome/ZmB73_5a_WGS \
   --no-novel-juncs genome/maize \
   fastq/2284_6063_7079_C3AR7ACXX_B_L1-3_CAGATC_R1.fastq.gz  \
   fastq/2284_6063_7079_C3AR7ACXX_B_L1-3_CAGATC_R2.fastq.gz >& B_L1-3.log &
tophat -p 7 -o L_L1-1 --transcriptome-index genome/transcriptome/ZmB73_5a_WGS \
   --no-novel-juncs genome/maize \
   fastq/2284_6063_7074_C3AR7ACXX_L_L1-1_CGATGT_R1.fastq.gz \
   fastq/2284_6063_7074_C3AR7ACXX_L_L1-1_CGATGT_R2.fastq.gz >& L_L1-1.log &
tophat -p 7 -o L_L1-2 --transcriptome-index genome/transcriptome/ZmB73_5a_WGS \
   --no-novel-juncs genome/maize \
   fastq/2284_6063_7077_C3AR7ACXX_L_L1-2_ACAGTG_R1.fastq.gz  \
   fastq/2284_6063_7077_C3AR7ACXX_L_L1-2_ACAGTG_R2.fastq.gz >& L_L1-2.log &
tophat -p 7 -o L_L1-3 --transcriptome-index genome/transcriptome/ZmB73_5a_WGS \
   --no-novel-juncs genome/maize \
   fastq/2284_6063_7080_C3AR7ACXX_L_L1-3_ACTTGA_R1.fastq.gz \
   fastq/2284_6063_7080_C3AR7ACXX_L_L1-3_ACTTGA_R2.fastq.gz >& L_L1-3.log &
tophat -p 7 -o S_L1-1 --transcriptome-index genome/transcriptome/ZmB73_5a_WGS \
   --no-novel-juncs genome/maize \
   fastq/2284_6063_7075_C3AR7ACXX_S_L1-1_TTAGGC_R1.fastq.gz \
   fastq/2284_6063_7075_C3AR7ACXX_S_L1-1_TTAGGC_R2.fastq.gz >& S_L1-1.log &
tophat -p 7 -o S_L1-2 --transcriptome-index genome/transcriptome/ZmB73_5a_WGS \
   --no-novel-juncs genome/maize \
   fastq/2284_6063_7078_C3AR7ACXX_S_L1-2_GCCAAT_R1.fastq.gz \
   fastq/2284_6063_7078_C3AR7ACXX_S_L1-2_GCCAAT_R2.fastq.gz >& S_L1-2.log &
tophat -p 7 -o S_L1-3 --transcriptome-index genome/transcriptome/ZmB73_5a_WGS \
   --no-novel-juncs genome/maize \
   fastq/2284_6063_7081_C3AR7ACXX_S_L1-3_GATCAG_R1.fastq.gz \
   fastq/2284_6063_7081_C3AR7ACXX_S_L1-3_GATCAG_R2.fastq.gz >& S_L1-3.log &
```

Multiple processors

General guidelines

- Do not run more processes/threads than CPU cores available on the machine
  - For large number of tasks, use script **`perl_fork_univ.pl`**
- Run only as many simultaneous processes as will **fit in memory** (RAM)
  - when in doubt, run a single process first and check its memory requirement (for example, using **`top`**)
- Programs heavy on I/O will compete for disk access if run in parallel – running too many simultaneously is not a good idea
- If available, use program's own multithreading options
- Using subset of input data, try to determine number of CPU cores which (for a given machine, input, and program options) gives the optimal speedup.

Exercises

Exercise: simple SNP-calling pipeline

Objective: align (simulated) Illumina reads to D. Melanogaster genome using **BWA** aligner and call variants using **samtools**

1. Copy the input data and shell script to your local working directory (replace my_id with your login ID):

```
mkdir /workdir/my_id
cd /workdir/my_id
cp /shared_data/Linux_workshop/pipeline_example.tgz .
tar -xzvf pipeline_example.tgz
```

2. Using commands like more, tail, head, wc,… to examine the sequence files (`genome.fa` – this is the reference genome; `reads.fastq` – these are the simulated Illumina reads), e.g.,

- `grep “>” genome.fa | wc` (will count chromosomes in genome)
- `wc reads.fastq` (the first number divided by 4 is the number of reads)

Exercise: simple SNP-calling pipeline

3. Open the file **`pipeline.sh`** in a text editor of your choice. Examine the structure of this file. Based on comments, identify commands corresponding to steps from slide "**Complex task example: SNP-calling**"

4. Run the pipeline in the background, saving any screen output to a log file. The run should take about 15 minutes.

```
cd /workdir/my_id
./pipeline.sh >& pipeline.log &
```

5. Use the **`top`**, **`ps`**, and **`ls`** commands to monitor the progress of the pipeline (processes and files).

6. List the generated output files and confront with script **`pipeline.sh`**

7. Using a text editor, examine the log file **`pipeline.log`**. Can you identify messages from individual commands in the script?

8. Using a text editor or text browsing commands (more, head, tail, etc) examine the alignment file (**`aln.sam`**) and final variant output file **`var.flt.vcf`**. You may want to look up the **SAM** and **VCF** format specifications (see http://samtools.sourceforge.net/ for quick reference).

Exercise: connect to your assigned workstation using VNC

- Go to “My Reservations” page
  http://cbsu.tc.cornell.edu/lab/lab.aspx , log in, click on “My Reservations” menu link
- Choose resolution (depends on your monitor)
- Click on “Connect VNC”
- Follow prompts to connect your VNC client to your VNC session
- Open terminal window in the VNC desktop by right-click on the desktop background and choosing “Open Terminal”.
- Disconnect (close VNC window) and then reconnect. Is the session still alive?