

Practical Linux Examples

- Processing large text file
- Parallelization of independent tasks

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

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

Estimate the percentage of sequencing reads in the FASTQ file that contains the adapter “AGATCGGAAGAGC”.

- Read file:
cat D7RACXX.fastq
- Select lines that contain the string
“AGATCGGAAGAGC”
grep AGATCGGAAGAGC
- Count the selected lines
wc -l

[illegible]

Estimate the percentage of sequencing reads in the FASTQ file that contains the adapter “AGATCGGAAGAGC”.

[illegible]

```
cat D7RACXX.fastq | grep AGATCGGAAGAGC | wc -l
```



```
cat DeD7RACXX.fastq | head -n 40000 | grep AGATCGGAAGAGC | wc -l
```

[illegible]

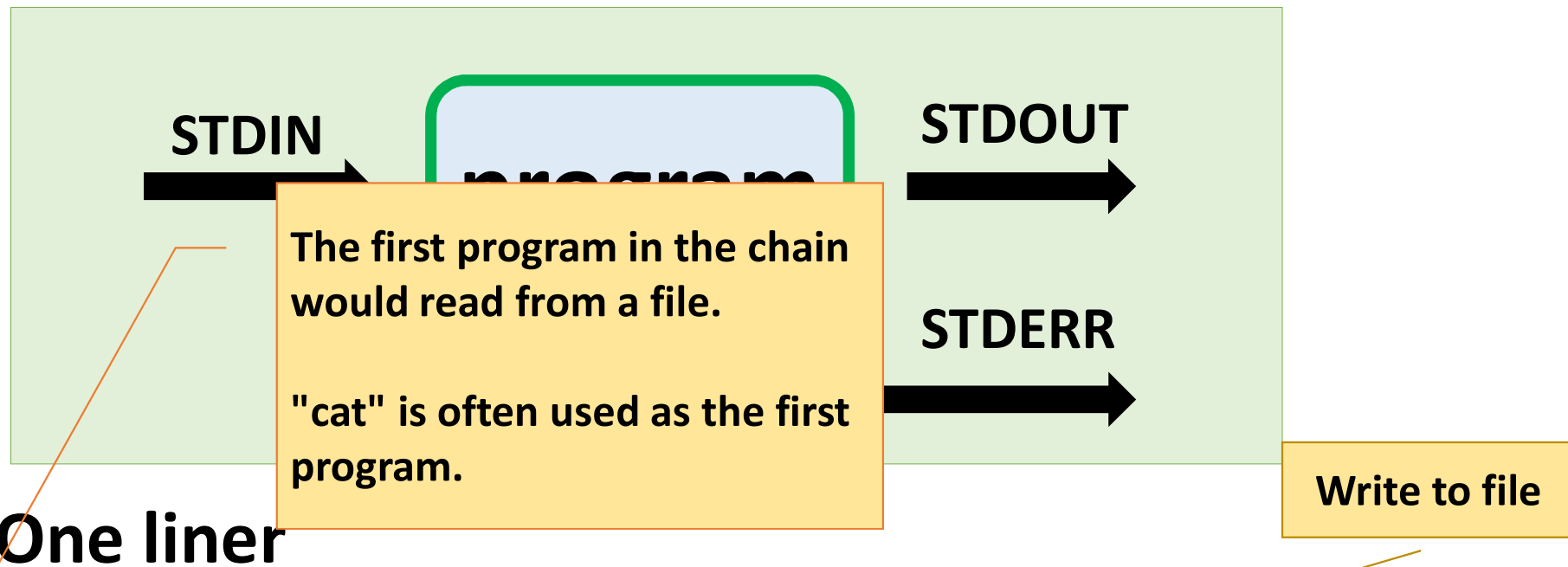
Estimate the percentage of sequencing reads in the FASTQ file that contains the adapter “AGATCGGAAGAGC”.

```
cat D7RACXX.fastq | \
head -n 40000 | \
grep AGATCGGAAGAGC | \
wc -l
```

Use “\” to separate the command into multiple lines

[illegible]

Three streams for a standard Linux program



One liner

Write to file

cat
inputfile

program 1

program 2

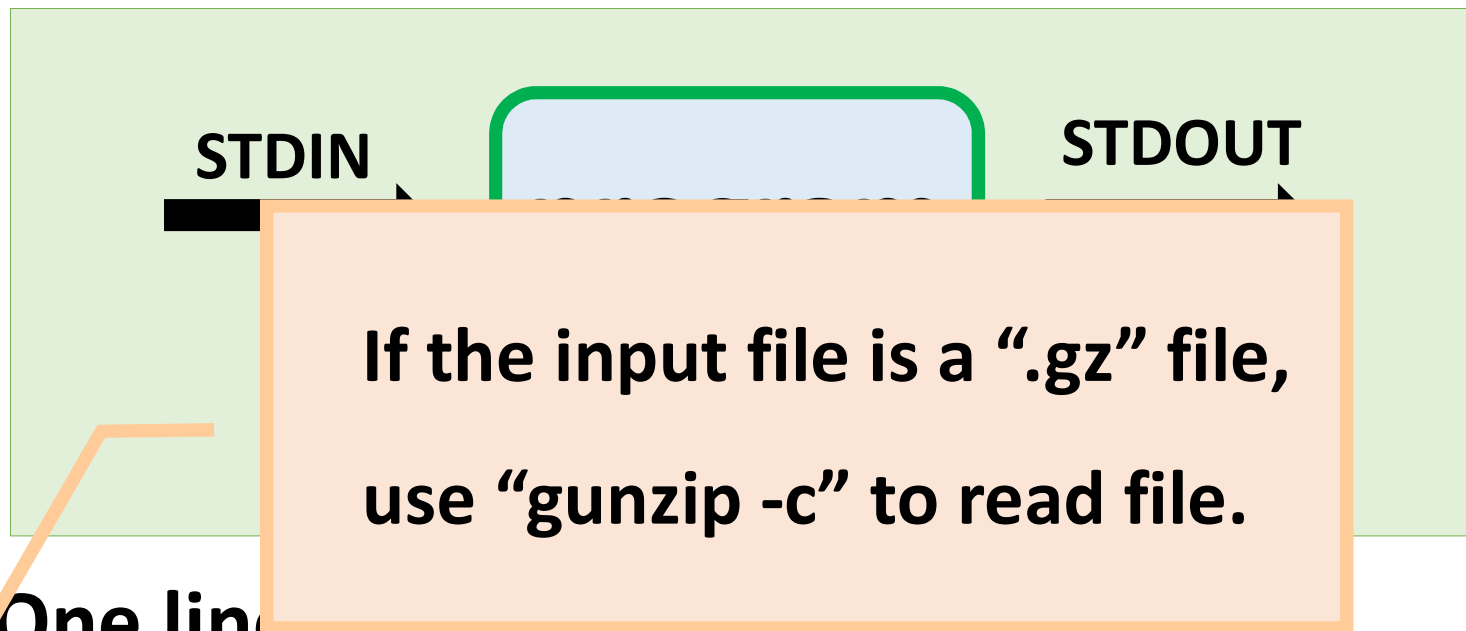
...

program n

>

Output
File

Three streams for a standard Linux program



grep

Search for a pattern and output
matched lines

```
$ cat mydata.txt
```

```
AAGATCAAAAAAGA  
ATTACGAAAAAGA  
ACCTGTTGGATCCAAAGTT  
AACTTTTCGACGATCT  
ATTTTTTTAGAAAGG
```

```
$ cat mydata.txt | grep '[AC]GATC'
```

```
AAGATCAAAAAAGA  
AACTTTTCGACGATCT
```


wc -l Count the number of lines

\$ cat mydata.txt

```
AAGATCAAAAAAGA
ATTACGAAAAAAGA
ACCTGTTGGATCCAAAGTT
AAACTTTCGACGATCT
ATTTTTTTTAGAAAGG
```

\$ cat mydata.txt | grep '[AC]GATC' | wc -l

2

sort

Sort the text in a file

\$ sort myChr.txt

```
Chr1  
Chr10  
Chr2  
Chr3  
Chr4  
Chr5
```

\$ sort -V myChr.txt

```
Chr1  
Chr2  
Chr3  
Chr4  
Chr5  
Chr10
```

\$ sort -n myPos.txt

```
1  
2  
3  
4  
5  
10
```

sort

Sort the text by multiple columns

```
$ sort -k1,1V -k2,2n myChrPos.txt
```

Chr1	12
Chr1	100
Chr1	200
Chr2	34
Chr2	121
Chr2	300

uniq -c

Count the occurrence of unique tags

```
$ cat mydata.txt
```

```
ItemB  
ItemA  
ItemB  
ItemC  
ItemB  
ItemC  
ItemB  
ItemC
```

```
$ cat mydata.txt | sort | uniq -c
```

```
1    ItemA  
4    ItemB  
3    ItemC
```

Mark sure to run
"sort" before "uniq"

Merging files:

cat f1 f2 VS **paste** f1 f2 VS **join** f1 f2

File 1:

Item1

Item2

File2:

Item3

Item4

cat File1 File2 > mergedfile1

Item1

Item2

Item3

Item4

paste File1 File2 > mergedfile2

Item1

Item3

Item2

Item4

* Make sure that that two files has same number of rows and sorted the same way. Otherwise, use “join”

join

Merging two files that share a common field

File 1:

Gene1	DNA-binding
Gene2	kinase
Gene3	membrane

File2:

Gene2	764
Gene3	23
Gene4	34

```
join -1 1 -2 1 File1 File2 > mergedfile
```

Gene2	Kinase	764
Gene3	membrane	23

```
join -1 1 -2 1 -a1 File1 File2 > mergedfile
```

Gene1	DNA-binding	
Gene2	Kinase	764
Gene3	membrane	23

cut

Output selected columns in a table

```
$ cat mydata.txt
```

```
Chr1 1000 2250 Gene1  
Chr1 3010 5340 Gene2  
Chr1 7500 8460 Gene3  
Chr2 8933 9500 Gene4
```

```
$ cat mydata.txt | cut -f 1,4
```

```
Chr1 Gene1  
Chr1 Gene2  
Chr1 Gene3  
Chr2 Gene4
```

sed

Modify text in a file

\$ cat mydata.txt

Chr1 1000 2250 Gene1

Chr1 3010 5340 Gene2

Chr1 7500 8460 Gene3

Chr2 8933 9500 Gene4

\$ cat mydata.txt | sed "s/^Chr//"

1 1000 2250 Gene1

1 3010 5340 Gene2

1 7500 8460 Gene3

2 8933 9500 Gene4

awk

Probably the most versatile function
in Linux

```
$ cat mydata.txt
```

```
Chr1 1000 2250 Gene1  
Chr1 3010 5340 Gene2  
Chr1 7500 8460 Gene3  
Chr2 8933 9500 Gene4
```

```
$ cat mydata.txt |\nawk '{if ($1=="Chr1") print $4}'
```

```
Gene1  
Gene2  
Gene3
```

A Good Practice:

Create a shell script file for the one liner

```
cat D7RACXX.fastq | \  
head -n 40000 | \  
grep AGATCGGAAGAGC | \  
wc -l
```

Run the shell script

```
sh checkadapter.sh
```

Debug a one-liner

```
gunzip -c human.gff3.gz | \
```



```
awk 'BEGIN {OFS = "\t"}; {if ($3=="gene") print $1,$4-1,$5}' | \
```

```
bedtools coverage -a win1mb.bed -b stdin -counts | \
```

```
LC_ALL=C sort -k1,1V -k2,2n > gene.cover.bed
```

```
gunzip -c human.gff3.gz | head -n 1000 > tmpfile
```

```
cat tmpfile | \
```

```
awk 'BEGIN {OFS = "\t"}; {if ($3=="gene") print $1,$4-1,$5}' | head -n 100
```

Many bioinformatics software support STDIN as input

```
bwa mem ref.fa reads.fq | samtools view -bS - > out.bam
```

Use "-" to specify input
from STDIN instead of a file

```
gunzip -c D001.fastq.gz | fastx_clipper -a AGATCG
```

```
..... | bedtools coverage -a FirstFile -b stdin
```


BEDtools - An Example

A file: Gene Annotation

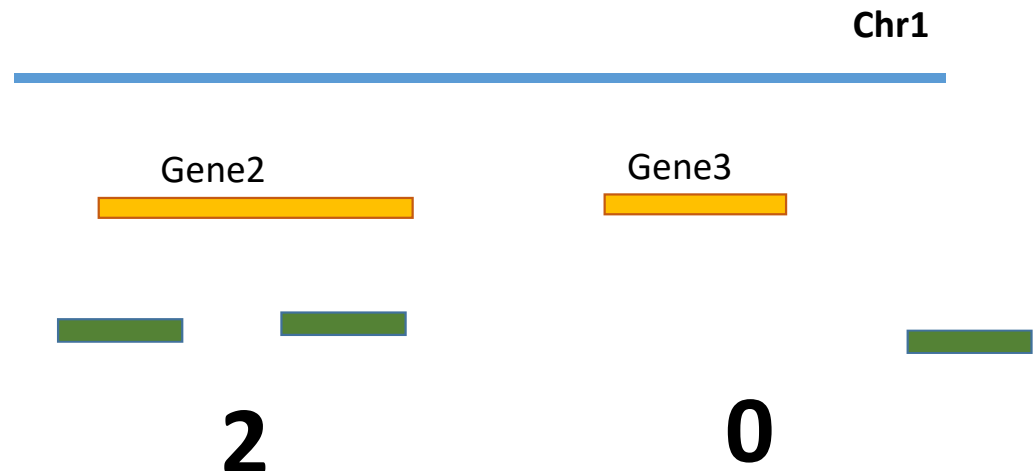
Chr1	1000	2250	Gene1	.	+
Chr1	3010	5340	Gene2	.	-
Chr1	7500	8460	Gene3	.	-
Chr2	8933	9500	Gene4	.	+
Chr2	12000	14000	Gene5	.	+

B file: Recorded Features

Chr1	200	300	Peak1
Chr1	4010	4340	Peak2
Chr1	5020	5300	Peak3
Chr2	8901	9000	Peak4

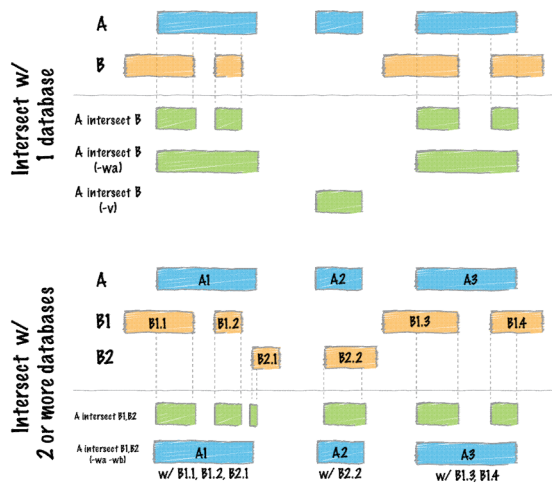
BEDtools coverage

		Number of overlapping peaks
...	Gene1	0
...	Gene2	2
...	Gene3	0
...	Gene4	1

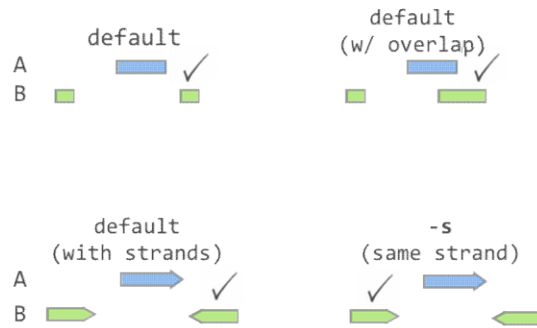


There Are Many Functions in BEDtools

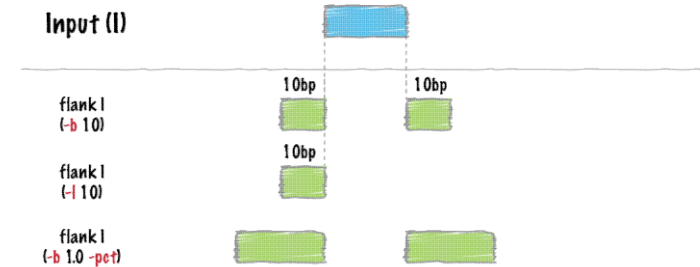
Intersect



Closest



Flanking



Other Tools that Work with Genomic Interval Files

SAM/BAM file

SAMtools, BAMtools, PICARD

VCF file

VCFTools, BCFtools

GFF3/GTF/BED

BEDtools, BEDOPS

Using multi-processor machines

All BioHPC Lab machines feature multiple CPU cores

- ☐ general (cbsum1c*b*): 8 CPU cores
- ☐ medium-memory (cbsummm*): 24 CPU cores
- ☐ marge-memory (cbsulm*): 64+ CPU cores

Using multi-processor machines

Three ways to utilize multiple CPU cores on a machine:

- ❑ Using a given program's built-in parallelization, e.g.:

```
blast+ -num_threads 8 [other options]
```

```
bowtie -p 8 [other options]
```

Typically, all CPUs work together on a single task. Non-trivial, but taken care of by the programmers.

- ❑ Simultaneously executing several programs in the background, e.g.:

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

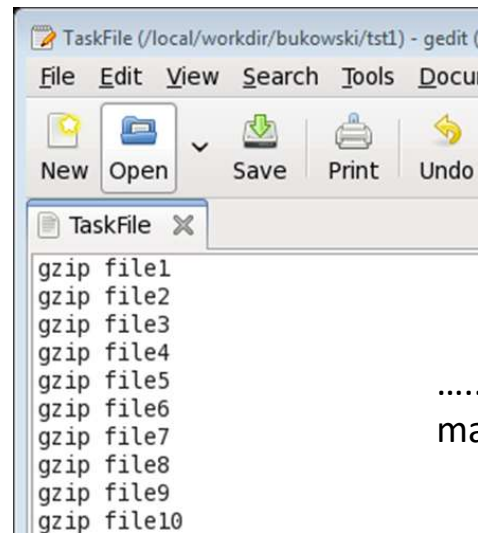
Multiple independent tasks

- ❑ If the number of independent tasks is larger than the number of CPU cores - use a “driver” program:

```
/programs/bin/perlscripts/perl_fork_univ.pl
```

Using perl_fork_univ.pl

Prepare a file (called, for example, **TaskFile**) listing all commands to be executed. For example,



.....number of lines (i.e., tasks)
may be very large

Then run the following command:

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

where **NP** is the number of processors to use (e.g., 10). The file “log” will contain some useful timing information.

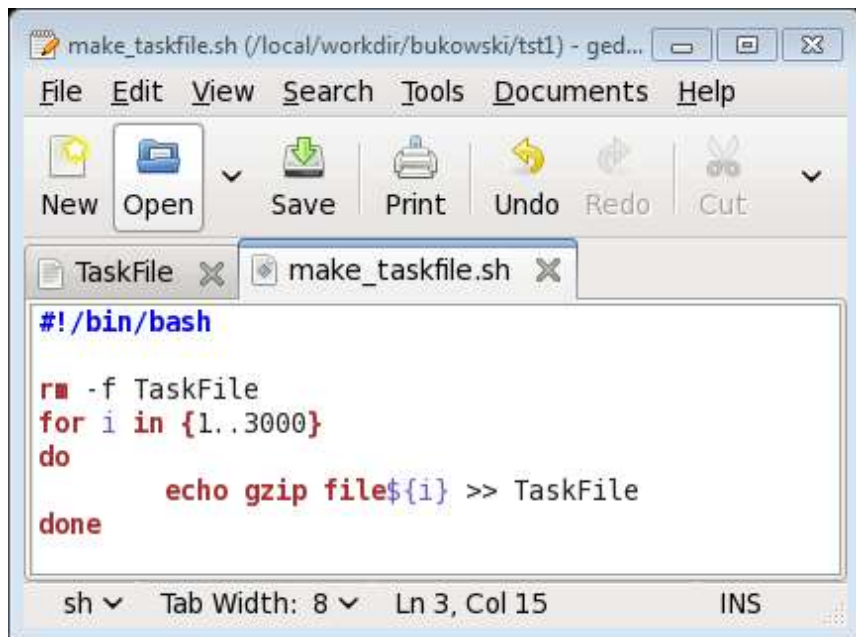
Using perl_fork_univ.pl

What does the script `perl_fork_univ.pl` do?

- ❑ `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**

Using perl_fork_univ.pl

How to efficiently create a long list of tasks? Can use “loop” syntax built into bash:



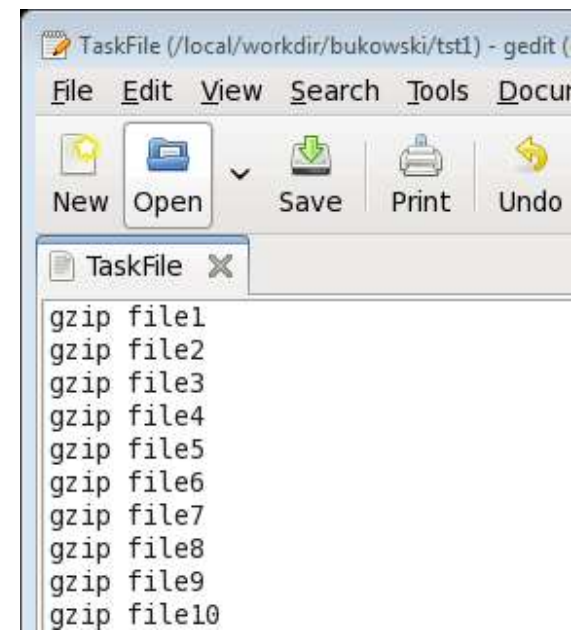
The screenshot shows a text editor window titled 'make_taskfile.sh (/local/workdir/bukowski/tst1) - gedit...'. The menu bar includes File, Edit, View, Search, Tools, Documents, and Help. The toolbar has icons for New, Open, Save, Print, Undo, Redo, and Cut. The text area contains the following bash script:

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

The status bar at the bottom shows 'sh', 'Tab Width: 8', 'Ln 3, Col 15', and 'INS'.



TaskFile



The screenshot shows a text editor window titled 'TaskFile (/local/workdir/bukowski/tst1) - gedit ('. The menu bar includes File, Edit, View, Search, Tools, and Docu. The toolbar has icons for New, Open, Save, Print, and Undo. The text area contains the following list of commands:

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

.....

Create and run a script like this, or just type directly from command line, ending each line with RETURN

How to choose number of CPU cores

Typically, determining the right number of CPUs to run on requires some experimentation.

Factors to consider

- total number of CPU cores on a machine: $NP \leq (\text{number of CPU cores on the machine})$
- combined memory required by all tasks running simultaneously should not exceed about 90% of total memory available on a machine; use **top** to monitor memory usage
- disk I/O bandwidth: tasks reading/write to the same disk compete for disk bandwidth. Running too many simultaneously will slow things down
- other jobs running on a machine: they also take CPU cores and memory!