PREREQUISITES

Make sure you already have an account in RCAC cluster (coates). You will receive a confirmation email about your account creation (unless you already have one) when your account has been created. The email will also have instructions on how to connect to the server. If you haven't received an email from ITAP then you probably don't have an account and you should contact the Bioinformatics core director about it immediately.

LOGGING IN

COATES: You can log onto its front-end/job-submission system (coates.rcac.purdue.edu) using your Purdue Career Account login and password. Logging into Coates requires an SSH client. There are several available for download on the Windows platform and Mac OS X/Linux have these built into their terminal application.

Microsoft Windows:

- PuTTY is an extremely small download of a free, full-featured SSH client.
- Secure CRT is a commercial SSH client which is freely available to Purdue students, faculty, and staff with a Purdue career account.

Mac OS X:

- The `ssh` command is pre-installed. You may start a local terminal window from "Applications->Utilities". Log in using

  ```
  ssh username@coates.rcac.purdue.edu
  ```

- MacSSH is another free SSH client.

Linux / Solaris / AIX / HP-UX / Unix:

- The `ssh` command is pre-installed. Log in using

  ```
  ssh username@coates.rcac.purdue.edu
  ```

QUEUES

Coates uses PBSPro for job scheduling and resource management. You will probably have access to the following queues.

bioinformatics - 48 cores, 720 hours
standby - 7856 cores, 4 hours
standby-e - 176 cores, 4 hours

FILE TRANSFER:

There are a number of ways to transfer data to and from RCAC systems. Which you should use depends on several factors, including the ease of use for you personally, connection speed and bandwidth, and the size and number of files which you intend to transfer.

SCP (Secure CoPy) is a simple way of transferring files between two machines that use the SSH (Secure SHell) protocol. You may use SCP to connect to any system where you have SSH (login) access. SCP is available as a protocol choice in some graphical file transfer programs and also as a command line program on most Linux, UNIX, and Mac OS X systems. SCP can copy single files, but will also recursively copy directory contents if given a directory name.

Command-line usage:

(to a remote system from local)
```
scp sourcefile username@coates.rcac.purdue.edu:somedirectory/
```

(from a remote system to local)
```
scp username@coates.rcac.purdue.edu:somedirectory/sourcefile
destinationfile
```

(recursive directory copy to a remote system from local)
```
scp sourcedirectory/ username@coates.rcac.purdue.edu:somedirectory/
```

Linux / Solaris / AIX / HP-UX / Unix:

- You will find the pre-installed "`scp`" command in your system.

Microsoft Windows:

- WinSCP is a full-featured and free graphical SCP and SFTP client.
- PuTTY also offers "pscp.exe", which is an extremely small program and a basic SCP client.
- Secure FX is a commercial SCP and SFTP client which is freely available to Purdue students, faculty, and staff with a Purdue career account.

Mac OS X:

- You will find the pre-installed "`scp`" command in your system. You may start a local terminal window from "Applications->Utilities".

GETTING STARTED

The data files required for this workshop are located in a public directory of the instructor's account. You need to have this in your home directory before you start the exercise 1. You can copy the directory by a simple command given below (just replace username with your Purdue login). You need to have an account in RCAC cluster to do this (coates.rcac.purdue.edu).

```
cp -r ~aseethar/WORKSHOP_FILES ~username/
```

Press enter

Note: the command is case sensitive

Once your cursor (command prompt) comes back to the original position, type

```
ls
```

Press enter

You should see "`WORKSHOP_FILES`" listed there.

UNIX EXERCISE 1

This exercise is designed to provide the basic skills required for working in the UNIX environment, using plenty of relevant examples, specifically for biologists. If you are using your personal computer, make sure that you have completed all the prerequisites before starting the exercise. This exercise will provide you information regarding navigation, files and directory creation/modification and some administrative things related to file permissions.

NAVIAGATION

This section will introduce you to some basic file/directory navigation and manipulation techniques.

TO KNOW THE PRESENT LOCATION OF YOUR COMMAND

```
pwd

/home/ba01/u143/username
```

Returns you the 'Present Working Directory'

This means, you are now working in the 'username' directory, which is located under 'u143' (parent) directory, which is located within 'ba01' directory and 'ba01' is within 'home' directory. The directory that you will be in after logging in is your home directory. You can also avoid writing the full path by using `'~'` in front of your username.

`~username` is same as writing `/home/ba01/u143/username`

Present directory is represented as `.` (dot) and parent directory is represented as `..` (dot dot)

CHANGING DIRECTORIES

To jump from one directory to another we use the `cd` (change directory) command.

```
cd ..
```
Changes your present location to the parent directory

```
cd DIRECTORY
```
This changes your location back to your `DIRECTORY`.

Step 1.1: Now change your directory to the 'WORKSHOP_FILES' directory present in your home directory.

NOTE: You can type in first few letters of the directory name and then press tab to auto complete rest of the name (especially useful when the file/directory name is long). This only works when there are unique matches for the starting letters you have typed. If there is more than one matching files/directories pressing tab twice will list all the matching names. You can also recall your previous commands by pressing up arrow or browse all your previously used commands by typing `history` on your terminal (typically last 500 commands will be saved in this file).

DIRECTORIES AND FILES

MAKING DIRECTORIES

To create a directory, `mkdir` can be used.

```
mkdir DIRECTORY
```

Unlike PC/Mac folders, here you can't have space in your directory name. Alternatively, you can also specify the path where you want to create your new folder.

Step 1.2: Make a new directory named 'FirstDirectory' within the 'WORKSHOP_FILES' directory. Then change your directory to the 'FirstDirectory'.

```
mkdir FirstDirectory
```

COPYING DIRECTORIES

To copy a file, `cp` command is used. When using this command you have to provide both source file and destination file.

```
cp SOURCE DESTINATION
```

You can also specify the absolute path of the source and/or destination file. To know more about any command you can use `'man'` command, which opens the 'manual' of the command you ask.

```
man cp
```

This opens the manual for the `cp` command. Take a look at the manual of `cp` command (use arrow keys to move top or bottom of the page). `OPTIONS` are optional parameters that can be used to accomplish more from the same command. For eg., using option `'-i'` with the regular `cp` command, you can always be sure that you are not overwriting the existing file while copying. The syntax for using the options will also be provided in the 'manual'. To exit, press `'q'`.

Looking at the man page for cp command, what options can be used to copy a directory (including all files within it)? _____

Is there any alternative method to view this manual page? _____

Step 1.3: Now change your directory back to the home directory. Create a copy of 'WORKSHOP_FILES' and name it as BACKUP_WORKSHOP). This will serve as a backup copy of all files that are required for the workshop (in case you accidentally modify the contents while working).

```
cp -r WORKSHOP_FILES BACKUP_WORKSHOP
```

MOVING DIRECTORIES

To move a file or a directory, `mv` command is used. Again, like the `cp` command you need to provide both source file and destination file.

```
mv SOURCE DESTINATION
```

Absolute path also works fine. Some of the options used by `cp` command also work with `mv` command. `mv` can also be used to rename files and directories

```
mv OLDNAME NEWNAME
```

Step 1.4: Rename 'WORKSHOP_FILES' as 'tutorials'.

```
mv WORKSHOP_FILES tutorials
```

VIEWING THE CONTENTS OF THE DIRECTORY

The contents of a dir can be viewed using `ls` (list) command.

`ls DIRECTORY` try this: `ls tutorials`

If no directory name is provided then `ls` will list all the contents of the present directory.

Like any other command, you can use absolute path or abbreviated path. There are also various options available for 'ls' command.

Some very useful options include:

`ls -l`
lists all the files in lengthy or detailed view

`ls -t`
lists all the files, sorted based on creation time

`ls -S`
lists all the files, sorted based on size

You can also combine these options together for getting more focused results.

Looking at the manual for ls, what option can you use to view hidden files in a directory (files starting with dot)? _____

Can you sort the list based on extension? How? _____

Step 1.5: Examine the contents of the 'tutorials' directory. Try options such as `-l, -t, -a` and `-x`. Also check if you can combine many options together (like `-la` or `-lh` etc) . Try these:

```
ls -l tutorials
ls -a
ls -1 tutorials
ls -lh tutorials
ls -t tutorials
```

CREATING AND EDITING FILES

`touch FILENAME`
Creates a new file in the present location

`nano FILENAME`
Like notepad, this text editor lets you edit a file.

Step 1.6: Create a new file named 'firstfile' inside the 'tutorials' directory. You can create using touch or using nano. Then add some contents (Your name and email address) to the 'firstfile' (using nano). After editing, press `Ctrl` and `X` to exit , then click to save changes and confirm the file name.

```
touch firstfile
nano firstfile
```

VIEWING CONTENTS OF THE FILES

There are various commands to print the contents of the file in bash. Most of these commands are often used in specific contexts. All these commands when executed with filenames displays the contents on the screen. Most common ones are

`less FILENAME` *try this:* `less AT_cDNA.fa`

Displays file contents on the screen with line scrolling (to scroll you can use 'arrow' keys, 'PgUp/PgDn' keys, 'space bar' or 'Enter' key). When you are done press `'q'` to exit.

`more FILENAME` *try this:* `more AT_cDNA.fa`
Like less command, also, displays file contents on the screen with line scrolling but uses only 'space bar' or 'Enter' key to scroll. When you are done press `'q'` to exit.

`cat FILENAME` *try this:* `cat AT_cDNA.fa`
Simplest form of displaying contents. It catalogs the entire contents of the file on the screen. In case of large files, entire file will scroll on the screen without pausing

`head FILENAME` *try this:* `head AT_cDNA.fa`
Displays only the starting lines of a file. The default is first ten lines. But, any number of lines can be displayed using `-n` option (followed by required number of lines).

`tail FILENAME` *try this:* `tail AT_cDNA.fa`
Similar to head, but displays the last 10 lines. Again `-n` option can be used to change this.

More information about any of these commands can be found in '`man`' pages (`man command`)

Step 1.7: Try using all these commands on the 'RefSeq.faa'. You are also welcome to try these commands on various other files that are present in the 'tutorials' directory. These commands don't change the contents of the file; they just display them on the screen.

DELETING FILES AND DIRECTORIES

To delete directories from the system, you can use `rmdir` (remove directory) command.

`rmdir DIRECTORY`
The directory should be empty before you use the `rmdir` command.

`rm FILE`
To delete a file `rm` command can be used

Some useful options include
`-r` recursively delete files
`-f` delete forcefully

`rm -rf DIRECTORY [DO NOT USE THIS NOW!]`
When you want to delete a folder, with all its content

Step 1.8: Delete the directory named 'delete_me' inside the 'tutorials' directory (to do this you may first want to delete the 'sample.txt' file inside this directory).

`cd delete_me`

```
rm sample.txt
cd ..
rmdir delete_me
```

COMPRESSING FILES

There are several options for archiving and compressing groups of files or directories. Compressed files are not only easier to handle (copy/move) but also occupy less size on the disk (less than 1/3 of the original size). In RCAC systems you can use zip, tar or gz for archiving and compressing files/directories.

ZIP compression/extraction

`zip OUTFILE.zip INFILE.txt`
Compress a file

`zip -r OUTDIR.zip DIRECTORY`
Compress all files in a directory into one archive file

`zip -r OUTFILE.zip . -i *.txt`
Compress all txt files in a `DIRECTORY` into one archive file

`unzip SOMEFILE.zip`
Decompress a file

Step 1.9: Zip 'AT_genes.gff' file located in the 'tutorials' directory. Check the file size before and after zip compression (Hint: use `ls -lh` to check file sizes)**.**

```
zip AT_genes.zip AT_genes.gff
```

Are the file sizes significantly different? *Y/N*

TAR utility saves many files together into a single archive file, and restores individual files from the archive. It also includes automatic archive compression/decompression options and special features for incremental and full backups.

`tar -cvf OUTFILE.tar INFILE`
archive `INFILE`

`tar -czvf OUTFILE.tar.gz INFILE`
archive and compress file `INFILE`

`tar -tvf SOMEFILE.tar`
list contents of archive `SOMEFILE.tar`

`tar -xvf SOMEFILE.tar`
extract contents of `SOMEFILE.tar`

`tar -xzvf SOMEFILE.tar.gz`
extract contents of gzipped archive SOMEFILE.tar.gz

`tar -czvf OUTFILE.tar.gz DIRECTORY`
archive and compress all files in a directory into one archive file

`tar -czvf OUTFILE.tar.gz *.txt`
archive and compress all `".txt"` files in current directory into one archive file

Step 1.10: Archive and compress the 'BACKUP_WORKSHOP' directory you created in step 1.3 (you can name it as 'backup.tar.gz' or anything you want)

```
tar -czvf backup.tar.gz BACKUP_WORKSHOP
```

GZIP compression utility designed as a replacement for compress, with much better compression and no patented algorithms. The standard compression system for all GNU software.

`gzip SOMEFILE`
compress SOMEFILE (also removes uncompressed file)

`gunzip SOMEFILE.gz`
uncompress SOMEFILE.gz (also removes compressed file)

Step 1.11: gzip the file 'AT_genes.gff' and examine the size. gunzip it back so that you can use this file for the later excercises.

```
gzip AT_genes.gff
ls -lh
gunzip AT_genes.gff.gz
ls -lh
```

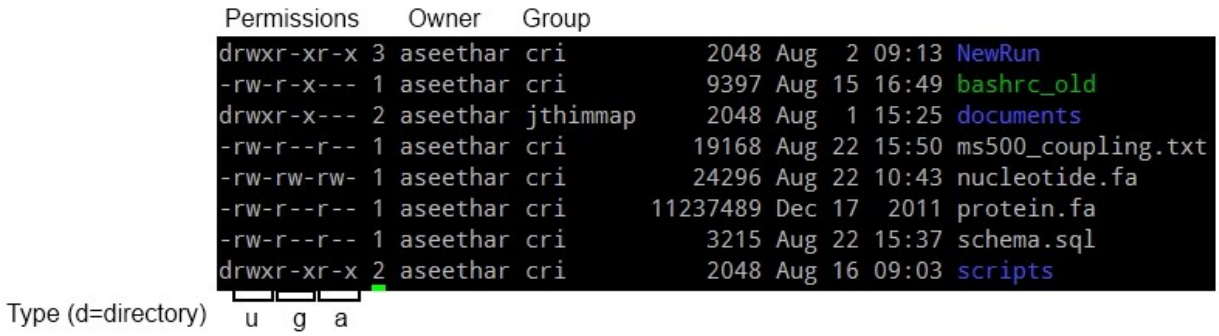
ADMINISTRATIVE COMMANDS

CHANGING PERMISSIONS

All files in the UNIX system will have a set of permissions which define what can be done with that file and by whom. (What = read (view contents), write (modify) and execute (run script) Whom=User (owner), group (that account belongs to) and everyone else). They are denoted as

PERMISSIONS		RELATIONS	
read	r	owner	u
write	w	group	g
execute	x	others	o
		all users	a

To look at the permissions for any file, you can list the files with `l` option (`ls -l`).



To set/modify a file's permissions you need to use the `chmod` command. Only the owner of a file can alter a file's permissions. The syntax:

```
chmod [OPTIONS] RELATIONS[+ or -]PERMISSIONS FILE
```

Add permissions : `chmod RELATIONS+PERMISSIONS FILENAME`

`chmod g+rwx FILENAME`	grants read, write and execute permissions for group
`chmod g+r FILENAME`	grants read permission for group
`chmod a+rwx FILENAME`	makes the file public (don't do this to any file/directory unless you want to share)

Remove permissions : `chmod RELATIONS-PERMISSIONS FILENAME`

`chmod g-wx FILENAME`	removes write and execute permissions for group
`chmod g-rwx FILENAME`	removes all permissions for group
`chmod a-rwx FILENAME`	removes all permissions for others
`chmod a-x FILENAME`	removes execution permissions for others

`OPTIONS` include

`-R` recursively (the permissions are applied to all the files, directories present inside the directory)

Step 1.12: Check the permissions for the files located in the 'tutorials' directory. Do

```
ls -l
```

What permissions does the group have on these files? _____

Which group does your account belong to? _____

UNIX EXERCISE 2

Second exercise on UNIX deals with more complex commands with their useful options and using multiple commands at a time. Make sure you understand all the commands from the previous exercise. This will also act as a guide for following the training session

FASTA FORMAT:

FASTA format is nothing but a simple text file containing either nucleotide sequences or protein sequences. An individual sequence always starts with a single line description of the sequence, followed by lines of sequence data. Description can be just an identification number or even blank (not recommended) but should always begin with a greater-than (">") symbol. The sequence is considered to be complete if another line starting with a ">" is encountered. The simplicity of FASTA format makes it easy to manipulate and parse sequences using text-processing tools and scripting languages like Python, Ruby, and Perl.

Some example FASTA format protein sequences are given below:

```
>gi|18403023|ref|NP_565747.1| splicing factor 3A subunit 2 [Arabidopsis
thaliana]
MDREWGSKPGSGGAASGQNEAIDRRERLRRLALETIDLAKDPYFMRNHLGSYECKLCLTLHNNEGNYLAH
TQGKRHQTNLAKRAAREAKDAPTKPQPLKRNVSVRRTVKIGRPGYRVTKQYDPELQQRSLLFQIEYPEIE
DNIKPRHRFMSSYEQKVQPYDKSYQYLLFAAEPYEIIAFKVPSTEVDKSTPKFFSHWDPDSKMFTLQVYF
KPTKPEPNKPQSAVGANGLPPPPPPPPHQAQPPPPPPSGLFPPPPPPMANNGFRPMPPAGGFGHPNM

>gi|224140247|ref|XP_002323495.1| predicted protein [Populus trichocarpa]
MDREWGSKPGSGGAASAQNEAIDRRERLRRLALETIDLAKDPYFMRNHLGSYECKLCLTLHNNEGNYLAH
TQGKRHQTNLAKRAAREAKDAPALPQPNKRKVNIRKTVKIGRPGYRVTKQFDPETKQRSLLFQIEYPEIE
DNTKPRHRFMSSYEQRIEANDKRFQYLLFSAEPYEIIAFKVPSTEIDKSTPKFFSHWDPDSKMFTLQLYF
KLKPPEANKPQSVAAANSTVPSQPPPPLPPQGLPAGSRPPPPPPMPASLPPPPPPAMANGPRPMPPGGAPP
APPPPPGGSGAMVNFTPGTQAGRPSSMLPPHGFLGQQMQGQTIRPPLLPPNMGQ
```

PIPES AND REDIRECTS

Many UNIX commands use some input file/data and display the output on the screen. This is feasible when the data being displayed is small enough to fit the screen or if it is the endpoint of your analysis. But for large data outputs, it is efficient to redirect to a file instead of screen. This can be done very easily in UNIX using > (greater than) or < (lesser than) or >> signs.

- '<' redirects the data to the command for processing
- '>' redirects the data from the command's output to a file. The file will be created if it is non-existing and if present it will overwrite the contents with the new output data (you will lose the original file).

- `'>>'` unlike `'>'` this redirection lets user append the data to an already existing file or a new file
- Another special operator `'|'` (called pipe) is used sometimes to pass the output from a command to another command (as input) before sending it to an output file or display.

Some *eg.*

```
cat FILE1 > FILE2
```
Creates a new file (file2) with same contents as old file (file1)

```
cat FILE1 >> FILE2
```
Appends the contents for file1 to file2, equivalent to opening file1, copying all the contents, pasting the copied contents to the end of the file2 and saving it!

```
cat FILE1 | less
```
Here, `cat` command displays the contents of the `file1`, but instead of sending it to standard output (screen) it sends it through the pipe to the next command `'less'` so that contents of the file are now displayed on the screen with line scrolling.

Step 2.1: The "Sequences" directory contains a number of files and each of these files contain a single FASTA formatted nucleotide sequence. Combine them all together to make a single file "sequences.fasta" using redirects.

```
cat *.fa >> sequences.fasta
```

will combine all .fa files into one.

REGULAR EXPRESSIONS

When working with the sequences (protein or DNA) we are often interested to see if a particular feature is present or not. This could be various things like a start codon, restriction site or even a motif. In UNIX all strings of text that follow some pattern can be searched using some formula called regular expressions. For *eg.* If you are looking for a particular motif in large number of sequences, then you can create a regular expression in UNIX and pick all the sequences with that motif relatively easily. Regular expression consists of normal and metacharacters. Commonly used characters include

Expression	Function
`.`	matches any single character
`$`	matches the end of a line
`^`	matches the beginning of a line
`*`	matches one or more character
`\`	quoting character, treat the next character followed by this as an ordinary character.
`[]`	matches one or more characters between the brackets
`[range]`	match any character in the range
`[^range]`	match any character except those in the range
`\{N\}`	match `N` occurrences of the character preceding (sometimes simply `+N)` where N is a number.
`\{N1,N2\}`	match at least `N1` occurrences of the character preceding but not more than `N1`
`?`	match 1 occurrence of the character preceding
`|`	match 2 conditions together, `\(this|that)\` *matches both this or that in the text*

For complete list, type `info regex` on your terminal.

Some examples related to nucleotide/protein sequences:

Patterns	Matches
`^ATG`	Find a pattern starting with ATG
`TAG$`	Find a pattern ending with TAG
`^A[T,G,C]G`	Find patterns matching either ATG, AGG or ACG
`TA[G,A]$`	Find patterns matching either TAG or TAA
`^A[T,G,C]G*TGTGAACT*TA[G,A]$`	Find gene containing a specific motif
`[X,N][M,P,R]_[0-9]\{4,9\}`	Find patterns matching NCBI RefSeq (*eg* XM_012345)
`\(NP|XP\)_[0-9]\{4,9\}`	Find patterns matching NCBI RefSeq proteins

Some common commands that can be used to manipulate text using regular expressions are `grep` (filters input against a pattern), `sed` (applies transformation after searching a pattern) and `awk` (manipulates data arranged in columns). We will discuss these commands in detail

GREP

The `grep` is one of the most useful commands in UNIX and it is commonly used to filter a file/input, line by line, against a pattern. For eg., to print each line of a file which contains a match for pattern.

```
grep PATTERN FILENAME
```

(Given no file, it reads from the standard input)

Like any other command there are various options available for this command. Most useful options include:

`-v`	inverts the match or finds lines NOT containing the pattern.
`--color`	colors the matched text for easy visualization
`-F`	interprets the pattern as a literal string.
`-H, -h`	print, don't print the matched filename
`-i`	ignore case for the pattern matching.
`-l`	lists the file names containing the pattern (instead of match).
`-n`	prints the line number containing the pattern (instead of match).
`-c`	counts the number of matches for a pattern
`-w`	forces the pattern to match an entire word.
`-x`	forces patterns to match the whole line.

With options, syntax is

```
grep [OPTIONS] PATTERN FILENAME
```

Some typical scenarios to use `grep`:

- Counting number of sequences in a multi-fasta sequence file
- Get the header lines of fasta sequence file
- Find a matching motif in a sequence file
- Find restriction sites in sequence(s)
- Get all the Gene IDs from a multi-fasta sequence files and many more.

Now let's use `'grep'` command to do some simple jobs with the sequences:

Counting sequences: By FASTA format definition, we know that number of sequences in a file should be equal to the number of description lines. So by counting `">"` in file, you can count the number of sequences. This can be done using counting option of the `grep (-c).`

```
grep -c ">" FILENAME
```

Step 2.3: Count the number of sequences `AT_cDNA.fa` and `RefSeq.faa`

```
grep -c ">" AT_cDNA.fa          _____
grep -c ">" RefSeq.faa          _____
```

If you are looking for information about the sequences, you can list all the headers (description lines) for the sequences using `grep`. Simply search for `">"` and `grep` will list all the description lines.

```
grep ">" FILENAME
```

```
grep ">" AT_cDNA.fa
```

Alternatively, you can send it to a file if you want to use it later or you can just pipe it to `'less'` or `'more'` command to scroll through it line by line or page by page.

```
grep ">" FILENAME > HEADERFILE.txt
```

```
grep ">" FILENAME | less
```

```
grep ">" AT_cDNA.fa | less
```

Use `'arrow'` keys to move up and down, press `'q'` to exit

See what kind of sequences are in `AT_cDNA.fa` file. Do they all seem to belong to same organism? yes/no Which organism? _____

Using grep you can also locate all the lines that contain a specific term you are looking for. This is very useful especially to look for a specific gene among a large number of annotated sequences.

```
grep "word or phrase to search" FILENAME
```

Step 2.4: Try searching for your favorite gene, to see if it is present in `AT_cDNA.fa` (this file contains all annotated sequences for *Arabidopsis thaliana*). Unlike Google or any search engines, only exact search terms will be identified, but you can ask grep to ignore cases while searching using `-i` option. Try these:

```
grep -i "transcription factor" AT_cDNA.fa
grep -i "TFIIIA" AT_cDNA.fa
```

You can also use this feature to see if your sequence of interest has a specific feature (restriction site, motif etc.,) or not. This can be performed better using `--color` option of the grep.

Go to the "sequences" directory, search for *Eco*R1 (GAATTC) site in the `'NT22.fa'` file, use the color option. Also, try looking for a C2H2 zinc finger motif in `RefSeq.faa` file (for

simplicity let's assume zinc finger motif to be CXXXCXXXXXXXXXXHXXXH. Either you can use dots to represent any amino acids or use complex regular expressions to come up with a more representative pattern. Try these:

```
grep --color "GAATTC" ./Sequences/NT22.fa
grep --color "C...C..........H...H" RefSeq.faa
```

You can also use grep command to exclude the results containing your search term. Say if you want to look at genes that are not located in chromosome 1, you can exclude it form your search by specifying `-v` option.

```
grep -i "transcription factor" AT_cDNA.f| grep -v "chr1"
grep -i "transcription factor" AT_cDNA.f| grep "chr1"
```

Notice the difference between above two commands.

Try to understand the following command lines (and record your results, where applicable):

```
grep -c -w "ATP" RefSeq.faa                    _____________
grep -c CGT[CA]GTG AT_cDNA.fa                  _____________
grep -l "ATG" ./sequences/*.fa
```

You can also try some regular expressions related to nucleotide/protein sequences provided earlier to see how it works.

SED

The `sed` command is a stream editor that reads one or more text files, makes changes or edits according to editing script, and writes the results to standard output. Most common editing script `sed` uses is to substitute text matching a pattern. The simple syntax for using sed is as follows

```
sed 'OPERATION/REGEXP/REPLACEMENT/FLAGS' FILENAME
```

Here, `/` is the delimiter (you can also use `_` (underscore), `|` (pipe) or `:` (colon) as delimiter as well)

`OPERATION` specifies the action to be performed (sometimes if a condition is satisfied). The most common and widely used operation is `s` which does the substitution operation (also useful operator is `y` which does transformation).

`REGEXP` and `REPLACEMENT` specify search term and the substitution term respectively for the operation that is being performed.

`FLAGS` are additional parameters that control the operation. Some common `FLAGS` include:

g	replace all the instances of REGEXP with REPLACEMENT (globally)
n	(n=any number) replace nth instance of the REGEXP with REPLACEMENT
p	If substitution was made, then prints the new pattern space
i	ignores case for matching REGEXP
w	file If substitution was made, write out the result to the given file
d	when specified without REPLACEMENT, deletes the found REGEXP

For brevity we only discuss sed command with respect to search and replace function. To do other things please refer to the man page of sed or the reference provided here http://www.grymoire.com/Unix/Sed.html#uh-47.

Some search and replace examples:
`sed 's/chr/chromosome/g' FILENAME` replaces ALL instances in a line
`sed '/MTF1/s/chr/chromosome/g' FILENAME`
replaces all instances in a line only if it contains 'MTF1'

Step 2.5: Try using replace function on 'AT_cDNA.fa' file (to change 'chr' to 'chromosome' and vice versa).

AWK

Unlike other UNIX commands awk is a structured language by itself. awk stands for the names of its authors Aho, Weinberger, and Kernighan. Many bioinformatics programs generate rows and columns of information. awk is an excellent tool for processing these rows and columns, and it is easier than most conventional programming languages.

The syntax for awk is:

```
awk 'PATTERN {ACTION}' FILENAME
```

awk then works by reading the input file one line at a time, matching the given PATTERN and performing the corresponding ACTION for the matches. If there is no PATTERN, then the ACTION will be performed on each line. But if there is no ACTION then the default ACTION (printing all lines) on the matching PATTERN will be performed (empty braces '{}' without any ACTION turns off default printing).

A simplest *eg*. would be

`awk '{print;}' FILENAME` *try* `awk '{print;}' AT_genes.gff`

Here, since there is no PATTERN, the print ACTION will be performed on each line (equivalent to `cat INFILE`).

Some inbuilt variables of awk include:

FS	Field Separator (default SPACE)
OFS	Output Field Separator (default SPACE)
NF	Number of Fields in the input
NR	Number of Records (lines) in the input
RS	Record Separator (default NEWLINE)
ORS	Output Record Separator (default NEWLINE)
FNR	File line number
N	N^{th} field of the line where N can be any number (eg. $0 = entire line, $1 = First field, $2 = second field and so on)

awk accepts all standard patterns (regular expression and expression) plus some special patterns

BEGIN	Special PATTERN that is executed before the INPUT is read
END	Special PATTERN that is executed after the INPUT is read
empty	*nonexistent* PATTERN that matches every input record

Some simple *eg.* using awk **(you can try these commands with 'AT_genes.gff' FILE)**

`awk NF FILE`	Deletes all blank lines
`awk 'NF > 0' FILE`	Deletes all blank lines
`awk 'NF > 4' FILE`	Prints the 4^{th} field of every line
`awk '$NF > 4' FILE`	Prints all lines with value of the 4^{th} filed > 4
`awk 'END { print $NF }' FILE`	Prints value of the last field of the last line
`awk 'NR==25,NR==100' FILE`	Prints lines between 25 and 100
`awk 'NR==50' FILE`	Prints 50^{th} line of input
`awk 'NR < 26' FILE`	Prints first 25 lines
`awk 'NR > 25' FILE`	Prints file after 25^{th} line
`awk 'END { print NR }' FILE`	Prints the last line of the file
`awk '{ print NF ":" $0 }' FILE`	Prints number of fields in front of every line
`awk '{ print FNR ":" $0 }' FILE`	Prints line number in front of every line
`awk '$5 == "abc123"' FILE`	Prints lines which have 'abc123' in 5^{th} field
`awk 'BEGIN { ORS="\n\n" }; 1' FILE`	Double spaces the file
`awk '{ print $1, $2 }' FILE`	Prints only 1^{st} and 2^{nd} field
`awk '{ print $2, $1 }' FILE`	Prints only 2^{nd} and 1^{st} field (swapping columns)
`awk '{ $2 = ""; print }' FILE`	Prints the file without 2^{nd} column
`awk '/REGEX/' FILE`	Prints all the lines having REGEX
`awk '!/REGEX/' FILE`	Prints all the lines not having the REGEX
`awk '/AAA|BBB|CCC/' FILE`	Prints all the lines having either AAA, BBB or CCC
`awk 'length > 50' FILE`	Prints line having more than 50 characters
`awk '/POINTA/,/POINTB/' FILE`	Prints section of file in between POINTA and POINTB

Try to understand the following command lines (and record your results, where applicable):

```
awk 'END { print $NF }' AT_genes.gff                    _________
awk 'NR==30,NR==35' AT_genes.gff.
awk 'NR==25' AT_genes.gff                               _________
awk 'NR<25' AT_genes.gff
awk 'END { print NR }' AT_genes.gff                     _________
awk '{ print NF ":" $0 }' AT_genes.gff > with_fields.txt.
awk '{ print NR ":" $0 }' AT_genes.gff > with_Line_num.txt.
awk '{ print $1, $3 }' AT_genes.gff
awk '{print $1, "\t", $3, "\t", $2}' AT_genes.gff
awk '{print $1,$2,$(NF-4),$(NF-3)}' AT_genes.gff.
awk '/Chr1/' AT_genes.gff.
```

TR

The tr utility in UNIX can translate or transliterate the input to produce a modified output. It uses 2 sets of parameters, and replaces occurrences of the characters in the first set with the corresponding elements from the other set.

```
tr [OPTIONS] "STRING1" "STRING2" <INFILE >OUTFILE
```

Options are

`-c`	complements the set of characters specified by string1
`-d`	delete occurrences of string1 (string2 not needed)
`-s`	squeeze repeats or multiple occurrences found in string1 will be replaced with one string2

Common uses of tr command are:

```
tr "a-z" "A-Z"        or        tr "[:lower:]" "[:upper:]"
```

Convert lower case to upper case (or upper to lower case)

```
tr -s '\n'
```

Convert each sequence of repeated newlines to a single newline

Files generated in both Mac and Windows OS will have a different 'newline' character (to mark the end of the line) that is not recognized by the UNIX OS. Similarly files generated in UNIX will have a different newline that can't be read in Windows or Mac OS. The 'tr' command provides an easy way to convert these 'newlines' to different forms.

```
tr '\r' '\n' <MAC.TXT >UNIX.TXT
```

Convert Mac text file to UNIX text file

```
tr '\n' '\r' <UNIX.txt >MAC.TXT
```
Convert UNIX text file to MAC text file

```
tr -d '\015' <WIN.TXT >UNIX.TXT
```
Convert Windows text file to UNIX text file

```
tr '\n' '\015'<UNIX.txt >WIN.TXT
```
Convert UNIX text file to windows text file

There are several utilities that can "mask" low complexity regions of the genomes such as repeats. They do that either by converting the bases/residues to lower case (soft masking) or converting them to N or X (hard masking). The public databases often store these soft masked genomes. When downloaded it might be useful to remove the masking, if your analysis doesn't require it (pattern searching etc.). It can be easily done by changes cases

```
tr "[ATGC]""[atgc]" <AT_cDNA.fa >AT_cDNA_tr.fa
```
Converts masking from the sequences and saves them in a new file

```
tr "[ATGC]""[AUGC]" <AT_cDNA.fa > AT_rna.fa
```
Converts cDNA to mRNA sequence and saves them in a new file

WORD COUNT

wc is another useful command that lets you count the number of words (and lines) in a file

`wc FILENAME` *try this* `wc AT_genes.gff`
This outputs both number of words as well as lines in a file.

`wc -l FILENAME` *try this* `wc -l AT_genes.gff`
Outputs only number of lines in file

Often these commands are "piped" with other commands to count certain things. Some *eg.*: Counting the number of files in a directory, counting the number of sequences etc.

Step 2.6: Count how many files with .fa extensions are present in 'sequences' directory.

```
ls Sequences| wc -l
```

SORT

sort command can be used to arrange things in a file. Simplest way to use this command is:

```
sort FILE1 > SORTED_FILE1
```

Useful options include

`-n`	numerical sort
`-r`	reverse sort
`-k N,N`	sort the Nth field (column), where N is a number. Sorting can also be done on the exact character on a particular filed *eg.* `-k 4.3,4.4` sorts based on 3rd and 4th character of the 4th field. Additionally you can supply additional `-k` for resolving ties.
`-t`	specify the delimiters to be used to identify fields (default is TAB) *eg.* `-t :` to use `':'` as delimiter

Step 2.7: The 'Sequences' directory consists of numerically labeled files. UNIX can sort either alphabetically or numerically (not both) and hence they are arranged in NT1.fa, NT10.fa, NT11.fa etc. In order to sort them in an easy to read way, try using

```
ls |sort -n -k 1.3,1.4
```

This command lists all the files in sequences directory and then passes it to sort command. Sort command then sorts it numerically but only using 3rd and 4th letters of the first field (file name)

Try using sort on AT_genes.gfffile

```
sort -r -k 1,1 AT_genes.gff
sort -r -k 3,3 AT_genes.gff
```

UNIQ

`uniq` command removes duplicate lines from a sorted file, retaining only one instance of the running matching lines. Optionally, it can show only lines that appear exactly once, or lines that appear more than once. uniq requires sorted input since it compares only consecutive lines.

```
uniq [OPTIONS] INFILE OUTFILE
```

Useful options include

`-c`	count; prints lines by the number of occurrences
`-d`	only print duplicate lines
`-u`	only print unique lines
`-i`	ignore differences in case when comparing
`-s N`	skip comparing the first N characters (N=number)

Step 2.8: Number each lines based on number of occurrences:

```
uniq -c ids.txt
```

Print only duplicated lines.

```
uniq -d ids.txt
```

Print only unique lines.

```
uniq -u ids.txt
```

COMPARING FILES

`diff` reports differences between files. A simple example for `diff` useage would be

```
diff [OPTIONS] FILE1 FILE2
```

Useful options include

`-b`	ignore blanks
`-w`	ignore white space (spaces and tabs)
`-i`	ignore case
`-r`	recursively compare all files (when comparing folders)
`-s`	list all similar files (when comparing folders)
`-y`	side by side comparison of files

The differences reported will be in the form of corrections that are required to change the first file to second file

Generate diffIDs.txt by comparing the differences between `ids_a.txt` and `ids_b.txt`

```
diff -y ids_a.txt ids_b.txt > diffIDs.txt
```

Are these files different?

`comm` command compares two sorted files line by line.

```
comm [OPTIONS] FILE1 FILE2
```

`-1`	suppress lines unique to `FILE1`
`-2`	suppress lines unique to `FILE2`
`-3`	suppress lines that appear in both files

Step 2.9: Compare the same files (`ids_a.txt` and `ids_b.txt`) again with 'comm' command and see how the outputs differ

```
comm -1 ids_a.txt ids_b.txt
comm -2 ids_a.txt ids_b.txt
comm -3 ids_a.txt ids_b.txt
```

DIVIDING FILES

CUT divides the file into several parts and displays selected columns or fields from each line of a file. Normally cut command requires how the fields are separated and what fields needs to be displayed.

`cut -d "," -f 2-4 FILE` displays columns 2,3 and 4 of a file separated by ","

`cut -d "|" -f 1,10 FILE` displays 1st and 10th columns of a file separated by "|"

`cut -f 1 FILE` displays 1st column of a file, assumes TAB as delimiter

SPLIT generates output files of a fixed size (bytes or lines). Useful when huge file needs to be processed. *eg.,*

```
split -d -l 100 filename suffix
```

here `-d` specifies numeric suffix only (`suffix00, sufix01, suffix02` *etc.*) while `-l` specifies number of lines in each file (100 in this case). If you want to split based on bytes, you can use `-b` option (*eg.* `-b 1k` or `-b 1m` for `1 KB` and `1 MB` respectively)

Note: You can join these files back using

```
cat suffix0[0-2] >> joinedfile
```

Step 2.10: Display only first column of the `'AT_genes.gff'` file using cut

```
cut -f 1 AT_genes.gff            (press ctrl + c to exit) or
cut -f 1 AT_genes.gff | less
```

Similarly, display 1st, 4th and 5th column of the `'AT_genes.gff'` file

```
cut -f 1,4,5 AT_genes.gff        (press ctrl + c to exit) or
cut -f 1,4,5 AT_genes.gff | less
```

Verify if all the columns in `'AT_genes.gff'` file has same number of entries in every field

```
cut -f 1 AT_genes.gff |wc -l
cut -f 2 AT_genes.gff |wc -l
cut -f 3 AT_genes.gff |wc -l
```

Split the file `'AT_genes.gff'` every 100,000 lines. Use `'gff_split'` as suffix for the files and use numerical suffix.

```
split -d -l 100000 AT_genes.gff gff_split
```

How many split files are generated: _____

```
ls gff_split* |wc -l
```

COMBINING FILES

PASTE prints lines consisting of sequentially corresponding lines of each specified file. eg.,

```
paste FILE1 FILE2 > FILE3
```

Combines the contents of file1 and file2, side by side generating a new file (file3).

Step 2.11: Combine columns of `ids_a.txt` and `ids_b.txt` files.

```
paste ids_a.txt ids_b.txt
```

How many columns do you see after combining? _____

JOIN combines two files based on the common field that is specified

```
join -t':' -1 N -2 N FILE1 FILE2
```

`-t':'` Specify field separator (here ":" but you can specify anything. Default is TAB)
`-1 N` Common field number (N) from the 1st file
`-2 N` Common field number (N) from the 2nd file

Step 2.12: Join columns based on column 1 in 'genes_a.gff' and column 3 in 'genes_b.gff'.

```
join -1 5 -2 3 genes_a.gff genes_b.gff
```

UNIX EXERCISE 3

This exercise mainly deals with using RCAC clusters for large scale data (Next Generation Sequencing analysis, Genome annotation, evolutionary studies etc.). These clusters not only have a large number of useful bioinformatics programs preinstalled but also have huge computational power to handle enormous data.

VARIABLES

When your account is setup, some standard variables (known as environment variables) that are specific to your account were created. These variables can be used to simplify your navigation (many environment variables specify storage locations and paths). Your login automatically defines these variables for you. Some standard variables are

Name	Description
`USER`	your username
`HOME`	path to your home directory
`PWD`	path to your current directory
`RCAC_SCRATCH`	path to scratch filesystem
`PATH`	all directories searched for commands/applications
`HOSTNAME`	name of the machine you are on
`SHELL`	your current shell (bash, tcsh, csh, ksh)
`SSH_CLIENT`	your local client's IP address
`TERM`	type of terminal or terminal emulator being used

To perform the action you need to use them with '$' sign in front. For example:

```
cd $RCAC_SCRATCH
```
Changes your directory from the current location to scratch space (additional space available for computing)

You can look up the values stored in these variables by using `echo` command
```
echo $VARIABLE_NAME
```

Additional RCAC specific commands include:

`myquota`	displays current space available on your home and scratch directories:
`qlist`	lists all queues that you can use
`findscratch`	to find the path to your RCAC scratch directory

PRE INSTALLED PROGRAMS

To use pre-installed applications you can use the `module` command. First configure it using following commands (please refer the wiki page for complete guidelines):

```
module load /opt/modules/versions/3.1.6
module use /apps/group/bioinformatics/modules
```

After that, you can use the `module load` command to access the software you want to use. For instance, to use FASTQC (program to check the quality of fastq reads of NGS) program,

```
module load fastqc
```

To check all available programs:

```
module avail
```

SUBMITTING JOBS

To submit a job to the Coates community cluster, you may use the portable batch system (PBS). If you run any jobs without the PBS then jobs will be executed on a front-end login host that is shared by all users. This will negatively impact everyone's ability to use Coates.

It is useful to keep a 'template' of a job submission file in your home directory, which can be modified every time you submit a new job. The template file should contain:

```
#!/bin/sh
#PBS -q myqueuename
#PBS -l select=1:ncpus=8
#PBS -l walltime=01:30:00
#PBS -N myjobname
cd $PBS_O_WORKDIR
module use /apps/group/bioinformatics/modules/
module load <program_name>
<your job>
```

Whenever you submit a job, you have to modify the number of nodes/processors, walltime, program name and your job script. Jobs can then be submitted using `qsub` command:
`qsub template_jobfile`
A sample job to check the quality of the reads obtained from a sequencing project is present in the `'jobfile'.` Let's run it on bioinformatics queue.
`qsub jobfile`
You will receive a confirmation "`1234567.coates-adm.rcac.purdue.edu`" where `1234567` is your job ID.

Once you have submitted the job script, you can view status of jobs by using following commands

`qstat -f yourjobid`	for information about your submitted job
`qstat -an1 yourqueuename`	current jobs running on queue you have submitted
`qstat -u yourusername`	list all the current jobs you are running on cluster
`qstat -a -u yourusername`	displays the status of your job

Additional resources:
http://www.rcac.purdue.edu/userinfo/resources/linuxcluster_pbspro/rcac_cluster_reference.pdf
http://www.rcac.purdue.edu/userinfo/resources/coates/userguide.cfm

Upon completion of the job, you will see many files in your working directory. Two of these files that start with your jobname are error log file (jobname.e1234567) and output log file (jobname.o1234567). The fastqc results for two reads will be in two separate folders (R1_fastqc and R2_fastqc). These folders are also saved as zip files by the program.

To view the results, change directory to one of the folder (R1_fastqc or R2_fastqc) and open 'fastqc_report.html' file. You can do this by

```
firefox fastqc_report.html
```

DOWNLOADING DATA

In order to start using the computational power of the coates, you need to first get the data there. If your data is already in your local computer, you can transfer them easily using WinSCP software or any other software (refer prerequisites). But if the data that you will be using is available in the public databases then you can directly get it from there using `wget` command.

To download data from NCBI Sequence Read Archive (SRA) or genomics core website

```
wget -O FILENAME "http://website.url"
```

As an example, we can download RNA-Seq sample from Illumina Genome Analyzer Run IIx from NCBI Sequence Read Archive (SRA).

```
wget -O SRR443170.fastq.gz
"http://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?cmd=dload&run_list=S
RR443170&format=fastq"
```

Note: this is a single line command

After completion, you might want to delete it from your home directory

```
rm SRR443170.fastq.gz
```