

# Linux for life scientists

**Introduction to Linux CLI** 

**Eline Turcksin** 



### **Course overview: (tentative)**

- 1. What is Linux?
- 2. Exploring the command line
- 3. Navigating the file system
- 4. Manipulating files and directories
- 5. File contents
- 6. Input, output and pipelines
- 7. Installing software & error handling
- 8. Bash scripting



# **0. TO DO beforehand**



### TO DO

- Install WSL (Windows users)
- Install homebrew (MacOS users)
- Install VSCode
- Request VSC account
- Check out terminal environment => will do together
  - <u>https://sandbox.bio/</u> to practice commands during course



### **Download course material**

- https://tinyurl.com/linuxcourse2024
- Click 'Download'

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# 1. Introduction



- It's difficult to install and work with
- It's totally different from Windows
- For everything you want to do, you need to write commands on a 'black screen'
- To work with Linux, you need to know/memorize so much commands, paths, settings
- These Linux/programming guys just seem like wizards



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## What is Linux?

#### Operating system (OS)

- An alternative to Windows (macOS)
- Graphic User Interface (GUI)
  - Similar in use as Windows

### Command-Line Interface (CLI)

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- Terminal
- "Under the hood"

### Availability

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- Many Linux distributions (=version)
- Usually free (not always e.g., RedHat)



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mark@linux-desktop:/tmp/tutorial\$ mkdir
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Try 'mkdir --help' for more information.
mark@linux-desktop:/tmp/tutorial\$ cd /etc ~/Desktop
bash: cd: too many arguments
mark@linux-desktop:/tmp/tutorial\$ ls
dir1 dir2 dir3
mark@linux-desktop:/tmp/tutorial\$

## What is Linux?

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### What is the CLI?

#### Command-Line Interface (CLI)

- CLI: typing commands
- Useful for accessing/working with data
  - Very powerful for data analysis
  - Learning curve
- System administration
- Plethora of CLI tools

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Linux/MacOS: terminal

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Windows: additional software needed

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- (MobaXterm: <a href="https://mobaxterm.mobatek.net/download-home-edition.html">https://mobaxterm.mobatek.net/download-home-edition.html</a>)
- Windows Subsystem for Linux <a href="https://learn.microsoft.com/en-us/windows/wsl/install">https://learn.microsoft.com/en-us/windows/wsl/install</a>

### What is the shell?

- A program
- Command line interpreter: waits for input and performs tasks
- Input language is a scripting language
- Shell provides access to 100s of commands
- Different shell programs exist
  - Linux systems: bash (Bourne Again Shell)
  - On MacOS: default is zsh, but making use of homebrew, also all Linux commands are available (though bash should be installed by default)



## **Linux Filesystem**

#### File System Tree

- 1 big directory structure
  - No separate disks (Windows C:\, D:\)
- Directory separators (path)
  - Linux = forward slash /
  - Windows = backwards slash \











### **Linux File**

- Paths
- Linux (GUI)
- ⊘ Recent ★ Starred A Home 🗖 Desktop Documents 🎜 Music Pictures

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➡ Videos

🛑 Trash

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A Home / Documents / datasets / emerson\_2017 / pubtcrs\_data\_v1 / pubtcrs\_data / clustering

**⊨** + **!** ●

: Q

## Linux Filesystem

#### File System Tree

- 1 big directory structure
  - No separate disks (Windows C:\ , D:\ )
- Directory separators (path)
  - Linux = forward slash /
  - Windows = backwards slash \
- Top level = root directory, or just /
- Subdirectories

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- /home/james
- Different folders = different purpose
  - /bin : holds programs (=binaries)
  - /tmp: to store temporary files
  - /home: user-specific folders

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#### Essential when using CLI (or when programming)!

- Learning to get the information you need is a major part of working in Linux/programming
- Sources:
  - Google (duh 🙂 )
    - Useful websites like StackOverflow (SO) (forums for variety of topics, including Linux commands)
    - But Google as a starting point is usually way to go
  - explainshell.com
    - Dissects bash command and explains every option

✓ ls(1) -ltr	
ist directory contents	
l use a long listing format	•
t sort by modification time, newest first	•
	1
r, -reverse reverse order while sorting	•







#### Sources

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- All (or almost all) commands have a 'help' option (either -h or --help):
  - \$ mkdir --help
    - Summary of most useful options
    - Usually not exhaustive

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- Syntax
  - Optional parts are in between []
  - ... to indicate it can be more than one

eate the DIREC	TORY(ies), if they do not already exist.
andatory argume	ents to long options are mandatory for short options too.
-m,mode=MOD	E set file mode (as in chmod), not a=rwx - umask
-p,parents	no error if existing, make parent directories as needed
-v,verbose	print a message for each created directory
-Z	set SELinux security context of each created directory
	to the default type
context[	=CTX1 like -Z, or if CTX is specified then set the SELinux
een een e	or SMACK security context to CTX
help	display this help and exit
version	output version information and exit
version	output version information and exit

or available locally via: info '(coreutils) mkdir invocation (exporter) pholmgren@ngsvm-pipelines:~/wes/aws\_utils\$

#### Sources

- Man pages
  - The full manual of a command
- Easiest with Google
  - Example: man <command>
  - <a href="https://linux.die.net/man">https://linux.die.net/man</a>
- CLI
  - \$ man <COMMAND>
  - Opens a reader
    - Scrollable: up/down arrows
    - To quit: press 'q'





#### Sources

- ChatGPT
- Advantage
  - Quick/elaborate explanations to your <u>specific</u> prompt
  - Works well for CLI but also when programming!
- Disadvantages
  - Sometimes false/inaccurate information
  - Need for double checking/testing



- Certainly! The given `sed` command performs several operations on a file. Let's break it  $\Box \ \Box \ \nabla$  down step by step:
- $1.~\mbox{'sed}\mbox{'}$  is a command-line utility for text stream editing in Unix-like operating systems.
- 2. The `-i` option is used to edit the file in-place, meaning the changes will be made directly to the file rather than printing the modified output to the terminal.
- 'in; s/,/\./g ; s/XY/X/g' ` is the set of sed commands enclosed in single quotes.
   Each command is separated by a semicolon (;). Let's break down each command:
  - `1n`: This command prints the first line of the file.
  - `s/,/\./g`: This command substitutes all occurrences of a comma (`, `) with a period
     (`.`) in the file. The `s/` indicates a substitution command, `, ` is the pattern to be replaced, `\.` is the replacement pattern, and `g` is the global flag to replace all occurrences in each line.
  - `s/XY/X/g`: This command replaces all occurrences of "XY" with "X" in the file. It follows a similar pattern as the previous substitution command.
- '\$file' is the file name provided as an argument to the 'sed' command. The '\$' sign is used to refer to the value of the variable "file" (assuming it has been previously defined).

In summary, the `sed` command edits the file in-place by performing three operations:

- 1. Prints the first line of the file.
- 2. Replaces all commas (`,`) with periods (`.`) throughout the file.
- 3. Replaces all occurrences of "XY" with "X" throughout the file.



#### Sources

- Make sure to double check
  - test code, critically inspect output
  - There might be easier solutions
  - Compare to other documentation

can you explain this sed command for me? sed -i '1n; s/,/\./g ; s/XY/X/g' '\$file'

- Certainly! The given `sed` command performs several operations on a file. Let's break it  $\Box \ \Box \ \bigtriangledown \ \nabla$  down step by step:
- $1.~\mbox{'sed'}$  is a command-line utility for text stream editing in Unix-like operating systems.
- 2. The `-1` option is used to edit the file in-place, meaning the changes will be made directly to the file rather than printing the modified output to the terminal.
- 3. `'1n; s/,/\./g ; s/XY/X/g'` is the set of sed commands enclosed in single quotes. Each command is separated by a semicolon (;). Let's break down each command:
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     occurrences in each line.
  - `s/XY/X/g`: This command replaces all occurrences of "XY" with "X" in the file. It follows a similar pattern as the previous substitution command.
- 4. `'**\$file**'` is the file name provided as an argument to the `**sed**` command. The `**\$**` sign is used to refer to the value of the variable "file" (assuming it has been previously defined).

In summary, the `sed` command edits the file in-place by performing three operations:

1. Prints the first line of the file.

2. Replaces all commas (`,`) with periods (`.`) throughout the file.

3. Replaces all occurrences of "XY" with "X" throughout the file.



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# 2. Exploring the command line



### **Command line basics**

- "\$": text preceding it = prompt; text followed by it = command
- Type a command, press enter to execute
- Autocomplete with TAB key
- Commands are
  - Case sensitive (myFile vs myfile)
  - Space sensitive (my file vs myfile)
- Mouse/clicking doesn't work! ③
  - Edit commands: use arrow keys (left and right)
  - Command history: arrow keys (up and down)

### **Command line basics**

- Copy pasting does not always work the same (depends on OS)
  - Instead of CTRL + C/V, try CTRL + shift + C/V
- No undo button => watch out with replacing/removing files/dirs.



### What are commands?

#### Command = specific instruction

- Given by the user, directed at the computer's operating system (windows, linux,...)
- Goal: let the computer perform a task or function

#### Examples

- Changing location
- Creating directories (folder), files, ...
- Change name of files, move things around
- Download software

### Why? (not GUI?)

- Faster, more powerful, can combine multiple actions in one command
- Not all tools/programs have a GUI



### **Anatomy of a command - simple**

#### Single command

- Some commands can be run by themselves, e.g.:
  - \$ cd = go to home directory (/home/user)
  - \$ 1s = list the contents of the folder you're currently in
- Most of the cases, they need some extra information...



### **Anatomy of a command - simple**

- Adding arguments: tell a command <u>what</u> to perform the action on
  - command + argument(s)
    - \$ cd /home/training
    - \$ ls my\_data/
  - How many arguments?
    - Depends on command, sometimes optional
      - E.g. \$ mkdir folder1 folder2
      - Creates 'subfolder1' and 'subfolder2'
    - Sometimes required
      - E.g. \$ mv important\_file.txt important\_folder
      - Moves file (first argument) into directory (second argument)



### **Anatomy of a command - intermediate**

- Adding options: modify a command's behaviour
  - command + options(s) + argument(s)
  - Options change behavior of commands
    - \$ ls <u>-1</u> /home/training
    - Print the content of the directory as before, but with more details (date created/modified, file size...)
  - Multiple options:
    - \$ ls -l -a -i
  - Options start with one or two -; e.g.:
    - \$1s -a : short option, usually one letter
    - \$1s --all: long option, usually a word
    - Both do the same



### **Anatomy of a command - options**

#### • Options:

- Short options can be grouped, long options can't
  - \$ ls -l -a -t -r  $\equiv$  ls -latr
  - \$ 1s --list --all : can't group, write in full
- Options don't always have both a short and a long version
  - Depends on the command
  - Check documentation (see later)
- Some options need a value (integer or string; number or text)
  - \$ head -n 15
  - \$ biotool --input /home/user/RNAseq\_data --output\_type csv



### **Anatomy of a command: options**

#### • Other example:

- \$ rm and \$ cp : when moving/copying folders you <u>need</u> to add the option -r
- Error often hints at the solution

```
training@ngscoursevm:~$ ls
folder1
training@ngscoursevm:~$ cp folder1 folder2
cp: -r not specified; omitting directory 'folder1'
training@ngscoursevm:~$ cp -r folder1 folder2
training@ngscoursevm:~$ ls
folder1 folder2
training@ngscoursevm:~$
```



### **The terminal**

▲ elinet@El.informatica: ~ × + ×	– o ×	● ● ●
<pre>elinet@ELinformatica:~\$ ls BIOMINA-24-005 BIOMINA-24-011 Python-3.12.0 Python-3.12.0.tgz R env_squiggle_linux.yaml get-docker.sh guppy_basecaller_log-2023-10-10_16-06-49.log mambaforge elinet@ELinformatica:~\$</pre>	project results snap software teaching test1 training wget-log work	john@Johns-MacBook-Pro ~ % sudo /Applications/Install\ macOS\ Sonoma.app/Con /Resources/createinstallmediavolume /Volumes/MyVolume Password: Ready to start. To continue we need to erase the volume at /Volumes/MyVolume. If you wish to continue type (Y) then press return: Y Erasing disk: 0% 10% 20% 30% 100% Making disk bootable Copying to disk: 0% 10% 20% 30% 40% 50% 100% Install media now available at "/Volumes/Install macOS Ventura" john@Johns-MacBook-Pro ~ %

### The terminal: command prompt

- eturcksin@DESKTOP1234:~\$ <here your command will go>
  - eturcksin = username
  - DESKTOP-1234 = name of device you're logged into (pc, server, ...)
  - ~ = current directory (after login, your home folder, so ~)
  - \$ : separator between the end of the prompt and the command your typing
    - Can differ between Linux flavors, can also be # or %
    - Command prompt is often abbreviated to "\$" instead of e.g. "eturcksin@DESKTOP1234:~\$"


## **Quick recap**





## Help with commands: shortcuts

#### Your cursor does not work in the terminal $\ensuremath{\mathfrak{O}}$

#### solution: arrows and shortcuts

Shortcut	Function
CTRL L	Prompt moves up; clears terminal
CTRL C	Kill process you're executing
CTRL arrows	Move along words instead of characters
CTRL A/CTRL E	Go to beginning/end of command
CTRL D	Exit terminal



## Help with commands: TAB completion

- The TAB key is very useful when typing in the CLI
  - Assists in completing command names and file/folder paths
  - Single TAB: completes your prompt when only one possibility is present

training@ngscoursevm:~/linux\_intro/philip\_holmgren\$ ls
data\_files file2
training@ngscoursevm:~/linux\_intro/philip\_holmgren\$ cd d

training@ngscoursevm:~/linux\_intro/philip\_holmgren\$ ls
data\_files file2
training@ngscoursevm:~/linux\_intro/philip\_holmgren\$ cd data\_files/

- Double TAB: when multiple possibilities exist, a suggestion list is given
  - Add the specific part, then TAB again to complete

- It works similar for commands
- Safes a lot of typing!





## Very useful: wildcards

#### • Wildcards = characters that can represent one or more other characters

- Useful to collect file/folders with a common name part
- Pattern searching
- \* : any number of any character
  - \$ 1s \*.fastq.gz (Lists all the files ending in fastq.gz)
- ? : any single character
  - \$ ls sample.chr?.bam
  - Will match sample.chr8.bam but nog sample.chr10.bam
- [0-9]/[a-z]/[A-Z]: a range of digits or letters
  - \$ ls sample.chr1[0-9].ba
  - Will match sample.chr10.bam, sample.chr11.bam ... sample.chr19.bam
- You can combine wildcards for more complex patterns

## Help with commands: good to know

#### Case sensitivity

- Linux commands are (mostly) all lower-case
- CD : will return the error 'CD: command not found'
- Common source of errors on CLI caused by using the wrong case

#### There is no undo in Linux

- Deleting/overwriting is permanent!
- 'Are you sure you want to delete this?' : you better <sup>(2)</sup>
- Be mindful of 'destructive' commands and check your commands before executing them
  - remove, overwrite...



# **Exercises part 2**



## **Terminal simulator**

#### Uniform terminal simulator to practice commands

- https://sandbox.bio/
- In nav header: Playgrounds > Terminal

Or directly to

https://sandbox.bio/tutorials/playground





- Intro demo
  - Let's create a little directory structure
    - \$ ls
    - \$ mkdir james
    - \$ ls
    - \$ cd james
    - \$ mkdir projects data Downloads
    - \$ ls
    - \$ cd projects
    - \$ mkdir RNA\_seq fwo paper
    - \$ touch fwo/summary.txt
    - \$ ls
  - Tip: try to find directories and files by using tab



### Some useful basic commands

- Is = list directory (folder) contents
- cd = change directories
- pwd = present work directory = current location
- touch = make a file
- mv = rename or move file/directory
- cp = copy file/directory
- rm = delete files/directories (!! Irreversible)



# 3. Navigating the filesystem



## Linux Filesystem: root

#### Root

- Only the 'superuser' can perform cmds
- = administrator space
- In practice: put "sudo" in front of cmds
  - Only works in a space where you have administrator privileges
- → Indicates administrator permission
- → Need to type password (not visible!)



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## Linux Filesystem: home

#### /home/user

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- Home contains directories with usernames
  - E.g. james, marie, guest, ...
- Similar to users in Windows
- In your own directory: free to execute cmds
- = space where you will perform your work
- Often abbreviated as ~
- \$ cd without arguments will bring you always to /home/user



## Navigating the file system

- Command cd (change directory)
- Current location: projects
- Want to go to 'fwo'
- Absolute path
  - Full path starting from root
  - \$ cd /home/james/fwo
- Relative path
  - Respective from the current directory
  - \$ cd fwo





## Navigating the file system

- Shortcuts for navigating
  - <u>Current directory</u>: . (single dot)
    - cd fwo  $\equiv$  cd ./fwo
  - <u>1 directory above</u>: .. (double dot)
    - cd .. => projects
    - cd ../../ => james
  - Your home folder: ~ (tilde)
    - cd /home/james  $\equiv$  cd ~ or cd ~/
    - cd /home/james/fwo  $\equiv$  cd ~/fwo
  - Previous folder: (hyphen)
    - cd -
  - Long paths are possible
    - cd ../data/fastq\_files





# **Exercises part 3**



#### Some useful basic commands

- Is = list directory (folder) contents
- cd = change directories
- pwd = present work directory = current location
- touch = make a file
- mv = rename or move file/directory
- cp = copy file/directory
- rm = delete files/directories (!! Irreversible)
- rmdir = remove (empty) directories



#### Exercise 1: start in project directory

- What is the difference between
  - \$ touch file1.txt .
  - \$ touch file2.txt
  - \$ touch ../file3.txt
  - \$ touch fwo/file4.txt

The difference between the commands is that the two first commands will make the files file1.txt and file2.txt in the project directory (your current directory). The third command will make the file3.txt in the directory above, here the james directory. The last command will make the file4.txt in the fwo directory which is located in your current directory.



#### Exercise 2: Start in the james directory

- 1. Go to /sys/module making use of a relative path
  - cd ../../sys/
- 1. Navigate to the kernel directory, located in sys. Make use of an absolute path. Then go back with `cd -`. Navigate again the that directory again with a relative path instead

cd /sys/kernel

cd –

cd kernel/

2. List the contents of the kernel directory

ls

3. Now without moving out of the kernel directory, list the contents of the power directory (it's a subdirectory of sys)

ls ../power

1. Make a new directory in the kernel directory. What happens?

mkdir: cannot create directory new\_dir: Operation not permitted

This is because you don't have the right permissions to make a directory in this place, because this is a directory in your root system, on your own computer you can do this with adding "sudo" upfront.



#### • Exercise 3.1

- Use the man pages, the --help option, ChatGPT to learn about the following commands (how and why to use), compare the answers
  - \$ pwd
    - Accessing the man pages: man pwd
    - Accessing the help page: pwd --help
  - \$ mv
    - man pwd
    - pwd --help
  - \$ cp
    - man pwd
    - pwd --help
- Use this information to complete the next exercises



#### • Exercise 3.2

- 1. Go to your home directory again, or the folders below if needed
- 2. Copy the summary.txt file from the fwo directory to the data directory cp james/projects/fwo/summary.txt james/data/
- Rename the summary.txt file in the data directory to boring\_exercise.txt cd james/data/

mv summary.txt boring\_exercise.txt



- Exercise 4
- The same as exercise 3 but you must stay in the Downloads directory
  - Navigate to the Downloads directory cd /root/tutorial/james/Downloads
  - Copy the summary.txt file from the fwo directory to the data directory cp ../projects/fwo/summary.txt ../data/
  - Rename the summary.txt file in the data directory to boring\_exercise.txt mv ../data/summary.txt ../data/boring\_exercise.txt



#### Exercise 5.1: 1s

- Create a file called .secret\_message.txt in the data directory touch data/.secret\_message.txt
- Can you find it using ls? Use info online to solve this No, you can solve this by adding the –a option
- 1. Delete the boring\_exercise.txt file rm data/boring\_exercise.txt
- 1. Remove the data directory
  - rmdir data/ -> doesn't work because the directory isn't empty
    - rm data/ -> doesn't work because it is a directory and not a file, this can be solved by adding the -r option
- 1. Problem/error? Try to google it!



#### Exercise 5.2: 1s

1. Try to find information about other 1s options

man ls

ls --help

2. What do all these columns in 1s -1 mean?



The first column is about permissions, the second one is the link count (the amount of links to the file or directory), then you have the owner's name and the group name. The fifth column is the size, then you have the date and hour of last modified. And as last the name of the file/directory



#### Exercise 6: naming constrictions

- Some characters are forbidden as a file or directory name ('/'), while some characters are considered bad practice ('\*', space, ':', '~', etc) and should be avoided.
- Why is '/' forbidden and why should '\*', space, ':' or '~' be avoided?
  - See comments for complete information about this
- If you do happen to have a file with '\*', space or '~' in the name, how could you
  quote it (i.e. refer to it) in the command line?

You can use "" or '' or put an / upfront of the character you don't to have interpreted.



## Intermezzo: WSL intro





#### Subsystem → Linux system inside of your Windows system

Access via terminal

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<sup>UB</sup> interactive command-line tools		Azure Cloud Shell
This PC - Documents		▶ Ubuntu





#### Subsystem Linux system inside of your Windows system

- Access via terminal
- This terminal will fulfill all your Linux hopes and dreams

🗼 elinet@ELinformatica: ~ 🛛 🗙	+ ~	
elinet@ELinformatica:~\$	Windows PowerShell	Ctrl+Shift+&
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	Azure Cloud Shell	Ctrl+Shift+"
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#### WSL

Access via file system



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- Other way also possible: access "Windows" files via WSL terminal
- To go to your C drive: \$ cd /mnt/c
- Navigate through your contents making use of Linux commands!



# 4. Manipulating files and directories



## Reminders

#### Linux is case-sensitive

- textfile.txt ≠ Textfile.txt
- If both present in same directory: different files

#### Avoid using spaces in file/folder names

- Spaces separates different parts of your CLI command
- Prone to make command mistakes
- Preferably use underscores \_ , hyphens or camelCase
  - full\_file\_name.txt
  - fullFileName.txt
- If you really want to use spaces, quote/escape your filename
  - "full file name.txt"
  - full\ file\ name.txt



## Files & directories: useful commands

#### touch, mkdir, rm (-r)

- touch: make an empty file
- mkdir: make a directory
- rm: delete a file
  - -r : to delete a directory, add this option



## File management: compressed files

#### Linux variant of zip: .gzip

- Commands gzip/gunzip
- \$ gzip textfile.txt > textfile.txt.gz
- \$ gunzip textfile.txt.gz > textfile.txt
- Read gzipped compressed files without unpacking
  - \$ zcat





## File management: archiving files

tar

- Makes a 'tarball' (.tar file): this is an archive file, holding multiple folders/files
- Optionally, compresses it (.tar.gz)
- Compress
  - \$ tar -zcf <COMPRESSED\_FILENAME>.tar.gz <DIRECTORY>
- Extract
  - \$ tar -zxf <COMPRESSED\_FILENAME>.tar.gz <DIRECTORY>
- -z: indicate the file is/will be zipped
- -c: compress mode
- -x: decompress mode
- -f: name of the source/destination (gzipped) tarball

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## File management: archiving files

#### Tar use?

 To transfer data, easier to transfer 1 file instead of (deep) directory structure

localadmin@ngscoursevm:/home/training/data\_files\$ ls PYTHON\_SCRIPTS VARIANT\_CALLING\_FILES bed\_files chr21.tar.gz emails.txt file.csv fileA.txt iqv\_bin Dummy\_Data ID\_SNP stdout.txt file.txt fileB.txt stderr.txt test\_file.txt Exome\_Data ID\_SNP2 R\_tutorial adapters chr21 dockers.txt fasta localadmin@ngscoursevm:/home/training/data\_files\$ tar czf ID\_SNP2.tar.gz ID\_SNP2 localadmin@ngscoursevm:/home/training/data\_files\$ ls Dummy\_Data ID\_SNP ID\_SNP2.tar.gz R\_tutorial adapters chr21 dockers.txt fasta file.txt fileB.txt stderr.txt test\_file.txt Exome\_Data ID\_SNP2 PYTHON\_SCRIPTS VARIANT\_CALLING\_FILES bed\_files chr21.tar.gz emails.txt file.csv fileA.txt igv\_bin stdout.txt localadmin@ngscoursevm:/home/training/data\_files\$ rm -rf ID\_SNP2 localadmin@ngscoursevm:/home/training/data\_files\$ tar xzf ID\_SNP2.tar.gz localadmin@ngscoursevm:/home/training/data\_files\$ ls Dummy\_Data ID\_SNP ID\_SNP2.tar.gz R\_tutorial adapters chr21 dockers.txt fasta file.txt fileB.txt stderr.txt test\_file.txt Exome\_Data ID\_SNP2 PYTHON\_SCRIPTS VARIANT\_CALLING\_FILES bed\_files chr21.tar.gz emails.txt file.csv fileA.txt igv\_bin stdout.txt localadmin@ngscoursevm:/home/training/data\_files\$

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## File management: archiving files

#### Echo

- Example: \$ echo "This is a koffiekoek"
- This command will "echo" the text that you give it
  - Here in the terminal This is a koffiekoek will appear
  - This is only outputted in the terminal and not saved anywhere

👌 marie	10:31:30 11/28/24			echo	"This	is	a	koffiekoek"
This is a	koffiekoek							
👌 marie	10:44:47 11/28/24		$\sim$	•				


# **Exercises part 4**



# Very useful: wildcards

#### • Wildcards = characters that can represent one or more other characters

- Useful to collect file/folders with a common name part
- Pattern searching
- \* : any number of any character
  - \$ 1s \*.fastq.gz (Lists all the files ending in fastq.gz)
- ? : any single character
  - \$ ls sample.chr?.bam
  - Will match sample.chr8.bam but nog sample.chr10.bam
- [0-9]/[a-z]/[A-Z]: a range of digits or letters
  - \$ ls sample.chr1[0-9].ba
  - Will match sample.chr10.bam, sample.chr11.bam ... sample.chr19.bam
  - To create files: use {0..9}
- You can combine wildcards for more complex patterns

# Some exercises

#### Intro

- Make a directory to work in, let's call it 'linuxcourse\_2024'
  - Put it in your personal (home) folder: /home/<user>/linuxcourse\_2024
  - Go into this directory mkdir /home/<user>/linuxcourse\_2024
  - cd /home/<user>/linuxcourse 2024
- Try the following commands and see what they do
  - \$ touch file.txt file2.txt
  - \$ tar -czvf file.tar.gz file.txt file2.txt
  - \$ ls -l
  - \$ rm file.txt file2.txt
  - \$ ls –la
  - \$ tar -xzvf file.tar.gz
  - \$ ls -la

#### Intermediate

- Make a subdirectory called 'exercise1' mkdir exercise1
- Make a file called 'test' with the text "This is a test" (reminder: echo) echo "This is a test" > test.txt
- Move the file into the directory 'exercise1' mv test.txt exercise1/
- Show all the content of the directory in long list form and make file sizes human readable Is -Ih exercise1/
- Make a tar file of the directory 'exercise1' tar -cvf exercise1.tar exercise1/



# Some exercises

#### Advanced

Make in one command 3 files with the following names file1A, file2A and file3B.
 Touch file{1A,2A,3B}

Make 10 files and they all are called file\_test1, file\_test2, ..., file\_test10
 Touch file\_test{1..10}

- Move all the file with an "A" in the 'exercise1' directory My file\*A exercise1/
- Write to the files with a "A" "this file is an A file"
  - Reminder: what directory are you in right now?
- echo "this file is an A file" > file1A; echo "this file is an A file" > file2A
- Remove the files file\_test1, file\_test2, ..., file\_test5
  - Try it using a wild card <a>!!! WATCH OUT WITH \$</a> rm \*

Rm file\_test{1..5}

Make a copy of the exercise directory and give it the name "exercise2"
 Cp exercise1/ exercise2

• Make an exercise3 directory

Mkdir exercise3/

- Move the rest of the test files (6-10) in 'exercise2'
- Mv file\_test{6..10} exercise2
- Finally, remove both these directories (2 and 3) rmdir exercise3/

Rm –r exercise2/

-> This must be done differently because the exercise3 directory is empty and the exercise2 directory contains files.



# Some exercises

#### Prepare downloaded data

- Data downloaded using intro (<u>https://tinyurl.com/linuxcourse2024</u>)
  - Is a .tar.gz file
- Move and extract the file from your Downloads folder to the working directory 'linuxcourse\_2024'

You can do this by the File Explorer on Windows

You can find your directory of your WSL by the following path \\wsl.localhost\Ubuntu\home\<your\_user\_name>

Or go in linux to your windows file system by the following path

Cd /mnt/c/Users/<username\_own\_computer>/Downloads/

• Will take a bit of time, just let it run



# 5. File contents



#### cat ('concatenate')

Prints one or more files to the screen

localadmin@ngscoursevm:/home/training/data\_files\$ cat fileA.txt
rowA
rowB
localadmin@ngscoursevm:/home/training/data\_files\$ cat fileB.txt
rowI
rowII
localadmin@ngscoursevm:/home/training/data\_files\$ cat file[A-B].txt
rowA
rowB
rowI
rowII
localadmin@ngscoursevm:/home/training/data\_files\$



#### head/tail [-<number\_of\_lines>]

- Prints the first/last lines of a file. Optionally, you can give the number of lines as an option
- Convenient for big files or when you only want the beginning/end of a file

training@ngscoursevm:~/linux\_intro/philip\_holmgren/data\_files\$ head -3 file.csv sample,raw.reads,aligned.reads,avg.bcov,p10x,p20x,Run 144928\_s12,29660708,42679036,44.716,0.05498,0.16008,3 138578,133206094,98900104,96.990,0.02883,0.05631,1 training@ngscoursevm:~/linux\_intro/philip\_holmgren/data\_files\$ tail -5 file.csv 120174,112289378,83869627,92.419,0.02732,0.05322,1 145314\_s13,21980408,34251706,35.385,0.09070,0.26475,3 143664\_s5,114484676,50864119,54.738,0.03909,0.10284,2 83477\_s2,22047074,34737358,36.760,0.06369,0.20144,3 142443\_s25,42113472,39925200,40.681,0.05439,0.17314,3 training@ngscoursevm:~/linux\_intro/philip\_holmgren/data\_files\$



#### - cut

- Print specific columns of a file
  - -f : number of column(s), can be a number, list or range
  - Default column separator/delimiter is TAB
    - -d : specify different delimiter
  - | head -4 : Linux pipe (ignore for now)



training@ngscoursevm:~/linux\_intro/philip\_holmgren/data\_files\$ cut -d, -f1,3 file.csv | head -4
sample,aligned.reads
144928\_s12,42679036
138578,98900104
135189,87900212
training@ngscoursevm:~/linux\_intro/philip\_holmgren/data\_files\$



#### sort

- Print the sorted lines of a file
  - Default sort: alphabetically
  - -n : numeric sort
  - -u : sort and removes duplicate lines
  - -k: sort on 1 or more columns
  - -V: version sort, use the numerical part to sort. Example: V1, V2, V3
  - -t: change the column delimiter (when using -k). Default is TAB

training@ngscoursevm:~/linux_intro/philip_holmgren/data_files\$ # -k2,2n : numerical sort on only the second columnk always needs a start and end column	
training@ngscoursevm:~/linux_intro/philip_holmgren/data_files\$ sort -k 2,2n file.txt   head -4	
sample raw.reads aligned.reads avg.bcov p10x p20x Run	
126895 131176 64548 0.066 1.00000 1.00000 1	
135038_s16 19646710 30228369 28.003 0.13701 0.38169 3	
133915_s4 21500824 33383572 31.840 0.09058 0.28122 3	
training@ngscoursevm:~/linux_intro/philip_holmgren/data_files\$ # -k2,2n -k1,1n : first sort on second, then on first column	
training@ngscoursevm:~/linux_intro/philip_holmgren/data_files\$ sort -t, -k2,2n -k1,1n file.csv   head -4	
sample,raw.reads,aligned.reads,avg.bcov,p10x,p20x,Run	
126895,131176,64548,0.066,1.00000,1.00000,1	
135038_s16,19646710,30228369,28.003,0.13701,0.38169,3	
133915_s4,21500824,33383572,31.840,0.09058,0.28122,3	
training@ngscoursevm:~/linux_intro/philip_holmgren/data_files\$	



#### • WC

- Get a word or line count for a file
  - Default returns 3 numbers: line count, word count, byte count
  - -I : only print line count (mostly used)
  - When given multiple files, it will give individual file and overall counts

localadmin@ngscoursevm:/home/training/data\_files\$ cat emails.txt john.doe@example.com alice\_smith@email.co.uk invalid-email bob\_jones123@gmail.com charlie-brown@yahoo.com eve@example.org invalid.email@.com localadmin@ngscoursevm:/home/training/data\_files\$ wc emails.txt 7 7 141 emails.txt localadmin@ngscoursevm:/home/training/data\_files\$ wc -l emails.txt 7 emails.txt



### Text patterns search

#### grep

- Print the lines of a file that match a certain word/pattern
- -i: case-insensitive search
- -n: show the matching line number
- -v: reverse, only show not matching lines
- -P : give a regular expression to match

training@ngscoursevm:~/linux\_intro/philip\_holmgren/data\_files\$ grep -n rowB test\_file.txt
2:rowB
training@ngscoursevm:~/linux\_intro/philip\_holmgren/data\_files\$ grep -ni rowb test\_file.txt
2:rowB
training@ngscoursevm:~/linux\_intro/philip\_holmgren/data\_files\$ grep -vni rowb test\_file.txt
1:rowA
3:rowC
4:rowD
5:row5
training@ngscoursevm:~/linux\_intro/philip\_holmgren/data\_files\$



# Text patterns search

#### Regular expressions (regex for short):

- Same idea as wildcards but more powerful
- Groups and ranges
  - . (dot) : any character
  - [0-9]: any digit
  - [a-zA-Z]: any letter (upper/lower case)
- Quantifiers
  - \* : zero or more characters
  - +: 1 or more characters
  - ?: 0 or 1 character
  - {m}: m characters ; {m,} : m or more characters ; {m,n} : number characters ranges from m to n
- Anchors
  - ^ : from beginning of the line
  - \$: at the end of the line
- Very extensive:
  - <u>https://cheatography.com/davechild/cheat-sheets/regular-expressions/</u>



# **Text patterns search: grep/regular expressions**

#### Grep examples

	localadmin@ngscoursevm:/home/training/data_files\$ cat dockers.t	xt			
	REPOSITORY	TAG	IMAGE ID	CREATED	SIZE
	391209680344.dkr.ecr.eu-west-1.amazonaws.com/aws_data_importer	1.4	1a9ec830c03b	22 hours ago	883MB
	basespace_to_aws	1.4	1a9ec830c03b	22 hours ago	883MB
	<none></none>	<none></none>	558acc514cd8	22 hours ago	1.08GB
	<none></none>	<none></none>	c1412423964d	22 hours ago	77.8MB
	<none></none>	<none></none>	4020ca978d13	22 hours ago	77.8MB
	391209680344.dkr.ecr.eu-west-1.amazonaws.com/upd-caller	dev	f62e3e6847af	4 weeks ago	4GB
	391209680344.dkr.ecr.eu-west-1.amazonaws.com/bedtools	2.29.2	73a68422e40a	5 weeks ago	112MB
	bt	dev	73a68422e40a	5 weeks ago	112MB
	391209680344.dkr.ecr.eu-west-1.amazonaws.com/swgs_cnv	1.1	fefd1210db1d	2 months ago	2.26GB
	swgs_cnv	1.1	fefd1210db1d	2 months ago	2.26GB
	swas_cnv	1.0	c31fcef2cff4	4 months ago	2.26GB
	391209680344.dkr.ecr.eu-west-1.amazonaws.com/swgs_cnv	1.0	c31fcef2cff4	4 months ago	2.26GB
	391209680344.dkr.ecr.eu-west-1.amazonaws.com/swas_ac	1.0	eq4643073be1	5 months ago	3.9GB
	swas_ac	1.0	ea4643073be1	5 months ago	3.9GB
	ubuntu	18.04	f9a80a55f492	5 months ago	63.2MB
	391209680344.dkr.ecr.eu-west-1.amazonaws.com/wes_cnv	0.4.1	de651d8a354f	5 months ago	4.92GB
	cromwell-dev	latest	6405fb36d05b	7 months ago	801MB
	391209680344.dkr.ecr.eu-west-1.amazonaws.com/wes_cnv	<none></none>	28db6f0e19e2	8 months ago	4.92GB
	201200680344.dkr.ecr.eu-west-1.amazonaws.com/swas.cnv	dev	79e9309d1a44	8 months ago	1.94GB
	nv	dev	79e9309d1a44	8 months ago	1.94GB
	680344.dkr.ecr.eu-west-1.amazonaws.com/bowtie2	2.5.1	295eef4f7951	8 months ago	664MB
	2	2.5.1	295eef4f7951	8 months ago	664MB
	ta importer	1 4	88ec7acaad97	8 months ago	967MR
	680344.dkr.ecr.eu-west-1.amazonaws.com/cutadapt	4.1	c58e97ada0b7	11 months ago	31.8MR
	nt	4 1	c58e97ada0b7	11 months ago	31.8MR
	ace to aws	1.3	fada8dc57aef	16 months ago	802MR
Z]{2.}' emails.txt	680344 dkr ecr eu-west-1 amazonaws com/aws data importer	1 3	fada8dc57aef	16 months ago	802MB
		22.04	27941809078c	17 months ago	77.8MB
		latest	27941809078c	17 months ago	77 8MB
		1 0	0007f75d5193	18 months ago	1 28GB
		focal	2h4cha85892a	20 months ago	72 8MP
	809758 dkr ecr eu-west-1 amazonaws com/winas-client	test	6a03c8e7e2be	24 months ago	12 QMR
	obs/so.uki.eci.eu-west-1.ulluzoiluws.coll/willigs-titerit	latest	6a03c8a7a2ba	24 months ago	12.9MD
	o /bac/anomacs	2021 3	5bdco2d86c60	2 voors ago	854MD
	ubustu	2021.5	f63181f10h2f	2 years ago	72 040
	angantwomon (yangmuli cuy	1 0 0	040070facao1	2 years ago	1 3700
	cmgantwerpen/varamplicnv	latest	e40079facae1	3 years ago	1 3760
	201200680311 dkn och overholstell anazonavs com/aatk	1 1 6 0	c8c508a2807a	3 years ago	2 7460
	251205000544.ukr.ecr.eu-west-1.umuzonuws.com/gutk	4.1.0.0	CoC330020070	5 years ago	2.7400
	rocatalithengscoursevill./holle/trathtng/data_ittess grep <hold< td=""><td>dockers.t.</td><td>559acc514cd8</td><td>22 hours ago</td><td>1 0900</td></hold<>	dockers.t.	559acc514cd8	22 hours ago	1 0900
	<pre>chone&gt;</pre>	<none></none>	5360CC314C06	22 hours ago	77 940
		<none></none>	4020cc078d12	22 hours ago	77.0MB
	<pre><number> 201200680244 dlum acm all wast 1 amazonawa com/was com/</number></pre>	<none></none>	20db660a10a2	22 nours ago	4 02CP
	local admin@nascourseym;/home/training/data_filesf_green_D_lame	<none></none>	Zouborver9ez	a montris ago	4.9208
	rocatal mengscoursevm. Thome/training/aata_filess grep -P */ <noi< td=""><td></td><td>EFReccE14-de</td><td>22 hours are</td><td>1 0900</td></noi<>		EFReccE14-de	22 hours are	1 0900
	<none></none>	<none></none>	5560CC514C08	22 nours ago	1.00GB
		<none></none>	402000078412	22 hours ago	77.0MB
	<none></none>	<none></none>	402000978013	zz nours ago	77.0MB
	tocaldammengscoursevm:/nome/training/aata_riles>				

bob\_jones123@gmail.com charlie-brown@yahoo.com eve@example.org invalid.email@.com localadmin@ngscoursevm:/home/training/data\_files\$ grep -P '[a-zA-Z0-9.\_%+-]+@[a-zA-Z0-9.-]+\.[a-zAjohn.doe@example.com alice\_smith@email.co.uk bob\_jones123@gmail.com charlie-brown@yahoo.com eve@example.org localadmin@ngscoursevm:/home/training/data\_files\$

> University of Antwerp Human Molecular Genetics Centre of Medical Genetics

ocaladmin@ngscoursevm:/home/training/data\_files\$ cat emails.txt

john.doe@example.com alice\_smith@email.co.uk invalid-email

# Before we do exercises, some tips

- Inspect your input data before you begin
  - Find patterns that you can use to parse or filter your wanted data
- When using regexes
  - Use the documentation or check online what the proper syntax is
  - You can copy-paste your regex in ChatGPT and just ask what's wrong with it
    - Or just ask it how to parse your data in the first place  $\ensuremath{\textcircled{\odot}}$



# **Exercises part 5**



# **Exercises**

- Go to linuxcourse\_2024/data\_files/fastq
  - Structure fastq file names
    - <SAMPLE\_NAME>\_S<INCREMENT\_NUMBER>\_<SEQUENCING\_LANE>\_<READ>\_001.fastq[.gz]
  - List all files ending in "fastq" ls –l \*.fastq
  - List all files with read 'R1' in file name Is –I \*R1\*
  - List all fastq files with Sample5 in file name Is Sample5\_\*.fastq
    - Try to avoid getting the files of sample Sample55
    - Now show the same files on screen using TAB completion
  - Using ls, can you find the largest file in this directory? Is –ISh (I = listform, S = sort, h = human readable)
    - Find out the ls option to sort on size
- Go to linuxcourse\_2024/data\_files/ID\_SNP
  - List all VCF files with filename in the range of 200 and 399 Is -- I {200...399}.vcf
    - Try to avoid .vcf.idx files
    - Use wildcards? Is it possible to get all files in 1 'range?' yes
      - If not, you can add additional items in your command (Is can take multiple arguments)
      - Is <range with wildcards> ... ...



# **Exercises**

- Go to linuxcourse\_2024/data\_files/fastq
  - How many lines does each fastq file of sample 5 contain? How much in total? wc -l \*fastq
  - Get the first line from every .fastq file? head -n 1 \*.fastq
  - Get all the lines (including line numbers) from all fastq files of sample 43 containing the string "@M00984".
     grep -n "@M00984" Sample43\_S91\_L001\_\*fastq
- Go to linuxcourse\_2024/ data\_files/ID\_SNP
  - Show the lines in 095.vcf that do NOT start with a "#". grep -v "^#" 095.vcf
    - Reminder: reverse grep
  - In samples 1-9, find the variants with depth of coverage equal to 1 (DP=1) grep "DP=1;" 00{1..9}.vcf
    - Did you restrict to DP=1 and ignored values between DP=10 and DP=19?
    - Check the line structure on how to do this
  - For the same files, can you get depth of coverage between 10 and 99? grep "DP=[1-9][0-9];" 00{1..9}.vcf
    - Tip: amount of digits = 2



# 6. Input, output and pipelines



# Data handling

#### • Up until now: print everything on the screen

• What if you want to write to a file?

#### Output streams

- Normal output (STDOUT)
- Error output (STDERR)
- Output redirection
  - STDOUT: >
  - STDERR: 2>

#### Appending

- STDOUT: >>
- STDERR: 2>>

localadmin@ngsco	oursevm:/home/tr	aining/data_file	s/ID_SNP:	\$ grep -	P "DP=∖d	{2,};" 0	<pre>0[1-9].vcf &gt; /tmp/my_stdout.txt</pre>
localadmin@ngsco	oursevm:/home/tr	aining/data_file	s/ID_SNP	\$ head -	5 /tmp/my	y_stdout	txt
001.vcf:chr1	67861520	rs2229546	С	Α	0	LowQual	AC=0;AF=0.00;AN=2;DB;DP=39;Dels=0.00;FS=0.000;Haplot
001.vcf:chr1	179520506	rs1410592	G	Α	1200.77		AC=2;AF=1.00;AN=2;DB;DP=33;Dels=0.00;FS=0.000;Haplot
001.vcf:chr2	169789016	rs497692	Т	C	296.77		AC=1;AF=0.500;AN=2;BaseQRankSum=2.068;DB;DP=42;Dels=
kSum=0.517	GT:AD:DP:GQ:PL	0/1:29,13:42:99	:325,0,8	23			
001.vcf:chr2	227896976	rs10203363	С	Т	671.77		AC=1;AF=0.500;AN=2;BaseQRankSum=-0.009;DB;DP=51;Dels
RankSum=1.083	GT:AD:DP:GQ:PL	0/1:26,25:51:99	:700,0,6	88			
001.vcf:chr3	4403767 rs28195	61 A	G	488.77		AC=2;AF=	=1.00;AN=2;DB;DP=19;Dels=0.00;FS=0.000;HaplotypeScore
localadmin@ngsco	oursevm:/home/tr	aining/data_file	s/ID_SNP	\$			

localadmin@ngscoursevm:/home/training/data\_files/ID\_SNP\$ grep -P "DP=\d{2,};" 00[1-9].vf 2> /tmp/my\_stderr.txt localadmin@ngscoursevm:/home/training/data\_files/ID\_SNP\$ head -5 /tmp/my\_stderr.txt grep: 00[1-9].vf: No such file or directory localadmin@ngscoursevm:/home/training/data\_files/ID\_SNP\$



# **Data handling**

#### Separate streams but can be redirected to the same file

- Syntax: > <FILE> 2>&1
  - grep something > /tmp/stdout.txt 2> /tmp/stderr.txt

localadmin@ngscoursevm:/home/training/data\_files/ID\_SNP\$ grep -P "DP=\d{2,};" 00[1-9].vf > /tmp/my\_stdout.txt 2> /tmp/my\_stderr.txt localadmin@ngscoursevm:/home/training/data\_files/ID\_SNP\$ grep -P "DP=\d{2,};" 00[1-9].vf >/tmp/my\_bothstreams.txt 2>&1 localadmin@ngscoursevm:/home/training/data\_files/ID\_SNP\$ cat /tmp/my\_bothstreams.txt grep: 00[1-9].vf: No such file or directory localadmin@ngscoursevm:/home/training/data\_files/ID\_SNP\$



# Data handling

#### Output of one command as input for another command

Pipe

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- Write between the different commands
  - \$ grep something <FILE> | head -5
- Can be any number of commands
  - Can make big one-liner scripts with this
  - Really convenient to check something quickly





# **Before we do exercices, some tips**

#### Again, investigate your input data

- Get a good feel of where you need to go
- Then work top-down
  - Example: get the second tab-separated column, then get the fourth element separated with ";", etc...
- Check the intermediate output from each command
  - Before you send it to another command
  - Best way to check which step has an issue if something goes wrong



# **Exercises part 6**



# **Exercises**

- Go to linuxcourse\_2024
  - Echo the text "Creating new file..." to the file "echo\_file.txt" echo "new file ..." > echo\_file.txt
    - Use the command 'echo' for this. First test out this command to see what it does
    - Check out the content of the file cat echo\_file.txt
  - Append the text "Adding a second line to my text file" to the same file echo "Adding a second line to my text file" >> echo\_file.txt
    - Check out the content of the file cat echo\_file.txt
- Go to linuxcourse\_2024/data\_files/fastq
  - How many files are in this directory? Print the results on the screen Is | wc -I
  - For how many samples do we have \*.fastq data? Is \*.fastq | wc -I
    - Use Is to first get the file names
    - Extract the sample from the file name somehow and remove duplicate sample names Is | cut -d\_ -f1
  - Copy the file "Sample43\_S91\_L001\_I1\_001.fastq" to a file called "copied\_file.fastq"

cat Sample43\_S91\_L001\_I1\_001.fastq > copied\_file.fastq

- Don't use the cp command but use output redirection to do the copy
- Verify by checking the content cat copied\_file.fastq

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# **Exercises**

- Go to linuxcourse\_2024/data\_files/VARIANT\_CALLING\_FILES
  - Count the number of files
    - Write the result to the file "file\_count.txt" ls | wc -l > ../file\_count.txt
    - Make it so that this file is made directly in your linuxcourse\_2024 folder
  - Get a size-sorted list of the files and print only the 3 largest files on the screen Is -SIh | head -n 3
    - Besides Is, can you find a Linux command that can calculate file sizes online? du -h \*
- Go to linuxcourse\_2024/data\_files/bed\_files
  - Get the last 1000 lines from the "large\_gene\_panel.bed" file and sort them on chromosome, and further sort on genomic start position tail -n 1000 large\_gene\_panel.bed | sort -k1,1V -k2,2n
    - Write the output to file "large\_file\_sorted.bed" in the same folder
    - tail -n 1000 large\_gene\_panel.bed | sort -k1,1V -k2,2n > large\_file\_sorted.bed
  - From "large\_gene\_panel.bed" select the exons corresponding to the AACS gene
  - tail -n 1000 large\_gene\_panel.bed | sort -k1,1V -k2,2n > large\_file\_sorted.bed
    - Write the genomic sorted output to file "AACS\_exons.txt" in the same directory
      - Are you sure you only selected AACS exons? If note, try to correct your output
    - On what chromosome is the AACS located? Write the solution to the screen on 1 line

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# 7. Software installation & error handling



# Ways to install & use software

- Imagine: experiment => fastq data
- You want to check the quality, don't know where to start
  - Google

A how to check quality of fastq data

ChatGPT

#### What can I help with?

how can check the quality of my fastq data? it's in fastq.gz format, and I've never worked with it before. I have installed WSL on my windows PC



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# Ways to install & use software

- FastQC: A quality control tool for high throughput sequence data
- Download options:
  - Package manager Linux (apt-get / dnf / yum ...)
  - Environment (Conda)
  - Container (Docker)
  - Manual (install binaries, add tool to \$PATH), least convenient but sometimes necessary
  - ...
- If you're using WSL: follow Linux installation instructions, not Windows



# Ways to install & use software: apt get

#### Installation with Linux package manager

- Installed on your "whole" system
- \$ sudo apt-get install fastqc
  - Why sudo?
  - Apt-get can also be yum, dnf, ...

#### I Not possible on HPC

→ Need sudo privileges
 (administrator) which we (luckily)
 don't have ☺

elinet@ELinformatica:~\$ sudo apt-get install fastgc Reading package lists... Done Building dependency tree... Done Reading state information... Done The following additional packages will be installed: libaec0 libapache-pom-java libargs4j-java libcommons-compress-java libcommons-io-java libcommons-jexl2-java libcommons-lang3-java libcommons-logging-java libcommons-math3-java libcommons-parent-java libfindbin-libs-perl libhdf5-103-1 libhtsjdk-java libjbzip2-java libjson-simple-java libngs-java libngs-sdk-dev libngs-sdk2 libsis-base-java libsis-base-jni libsis-jhdf5-java libsis-jhdf5-jni libsnappy-java libsnappy-jni libsnappy1v5 libsz2 Suggested packages: libxz-java libcommons-io-java-doc libcommons-lang3-java-doc libavalon-framework-java libcommons-logging-java-doc libexcalibur-logkit-java liblog4j1.2-java picard-tools libjson-simple-doc The following NEW packages will be installed: fastqc libaec0 libapache-pom-java libargs4j-java libcommons-compress-java libcommons-io-java libcommons-jexl2-java libcommons-lang3-java libcommons-logging-java libcommons-math3-java libcommons-parent-java libfindbin-libs-perl libhdf5-103-1 libhtsjdk-java libjbzip2-java libjson-simple-java libngs-java libngs-sdk-dev libngs-sdk2 libsis-base-java libsis-base-jni libsis-jhdf5-java libsis-jhdf5-jni libsnappy-java libsnappy-jni libsnappy1v5 libsz2 0 upgraded, 27 newly installed, 0 to remove and 93 not upgraded. Need to get 9407 kB of archives. After this operation, 16.2 MB of additional disk space will be used. Do you want to continue? [Y/n]



# Ways to install & use software: conda

#### Conda allows for package and environment management

- <u>Environment management</u>: can create smaller environments within your system to download specific software in. Only when you're present in the environment, you can run the tools you downloaded.
- <u>Package management</u>: easy to download specific versions of packages/software, needed for a specific project
- Rule of thumb: for each new project, new conda environment
- Use of environments is good practice, to keep your general space "clean": avoid unwanted dependency errors



# Ways to install & use software: conda

- 1. Download conda (we're not doing this right now) only first time
- 2. Create environment
- 3. Activate environment
- 4. Download packages/tools
  - Either you download the necessary tools one by one, by using
    - \$ conda install <package name>
    - \$ conda install <package1> <package2>
  - Or you give it a file to build an environment from (.yaml) → important for HPC
- 5. Do your analysis
- 6. Deactivate environment

# Ways to install & use software: conda (locally)

#### Creating environment

#### elinet@ELinformatica:~\$ conda create -n project\_name

Retrieving notices: ...working... done Channels:

- conda-forge
- bioconda
- defaults
- Platform: linux-64

Collecting package metadata (repodata.json): done Solving environment: done

```
==> WARNING: A newer version of conda exists. <==
    current version: 24.5.0
    latest version: 24.11.0</pre>
```

Please update conda by running

\$ conda update -n base -c conda-forge conda

## Package Plan ##

environment location: /home/elinet/mambaforge/envs/project\_name

# Proceed ([y]/n)? y Preparing transaction: done Verifying transaction: done Executing transaction: done # # To activate this environment, use # \$ conda activate project\_name # # To deactivate an active environment, use # # \$ conda deactivate elinet@ELinformatica:~\$



# Ways to install & use software: conda (locally)

#### List existing envs, activate environment

<pre>elinet@ELinformatica:~\$ # conda environments: #</pre>	conda env list
base	/home/elinet/mambaforge
project_name	/home/elinet/mambaforge/envs/project_name
ргокка	/home/elinet/mamba+orge/envs/prokka
virtus	/home/elinet/mambaforge/envs/virtus

elinet@ELinformatica:~\$ conda activate project\_name
(project\_name) elinet@ELinformatica:~\$



# Ways to install & use software: conda (locally)

- Install the tool(s) you want to install
- Channels:
  - Large repositories
  - Contain packages
  - E.g. bioconda repository, contains lots of interesting software for bioinformatics purposes
- Check if command is available
- Done!



	<pre>(project_name) elinet@ELinformatica:~\$ conda install fastqc</pre>
	- conda-forge
	- bioconda
	- defaults
	Platform: linux-64
	Collecting package metadata (repodata.json): done
	Solving environment: done
	==> WARNING: A newer version of conda exists. <==
	current version: 24.5.0
	Latest Version: 24.11.0
	Please update conda by running
	\$ conda update -n base -c conda-forge conda
S	
	## Dackage Dlan ##
	"" rachage rian ""
	environment location: /home/elinet/mambaforge/envs/project_name
	added / updated specs:
	- fastqc
	The following packages will be downloaded:
	The foctowing packages will be downloaded.
	package build

fastgc-0.12.1

fontconfig-2.15.0

hdfd78af

11.1

259

KB

bioconda

conda-forge

# Ways to install & use software: conda (HPC)

- On HPC => conda discouraged, because it creates lots of small files
- Still need software?
  - Check if it's already installed (in doubt, contact CalcUA)
  - Not installed? Create a .yaml with your packages needed!


## Ways to install & use software: conda (HPC)

- How to make a .yaml
  - Create a new file
  - Type the following info:
    - Name of environment
    - Channels to look in (usually always these three)
    - Dependencies = packages
       = software you need
    - Can define version (see python)
  - Save file by naming it name.yaml

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Iniversity of Antwerp Human Molecular Genetics Centre of Medical Genetics name: rnaseq\_env
channels:

- bioconda
- conda-forge
- defaults
- dependencies:
  - python=3.9
  - fastqc
  - trimmomatic
  - hisat2
  - samtools
  - featurecounts
  - star
  - salmon
  - r-base=4.2
  - r-essentials
  - bioconductor-deseq2
  - multiqc
  - pandas
  - matplotlib
  - numpy

- # Python version
- # Quality control for raw sequencing reads
- # Adapter trimming and quality filtering
- # RNA-seq alignment tool
- # Manipulation of SAM/BAM files
- # Gene-level quantification
- # Spliced aligner for RNA-seq data
- # Transcript-level quantification
- # R for downstream analysis
- # R packages like ggplot2, dplyr, etc.
- # Differential expression analysis
- # Aggregates QC reports
- # Python library for data analysis
- # Python library for plotting
- # Python library for numerical computing

## Ways to install & use software: conda (HPC)

#### How to make a .yaml

- OR: build an environment with all your wanted software on your own PC
- Then export your environment to a yaml
- \$ conda env\_name export > environment.yml
- Then use this environment.yml in the next step



## Ways to install & use software: conda (HPC)

- This .yaml is then used to create a <u>container</u> using the hpc-wrappercontainer command (see documentation CalcUA)
- Container?!
  - Even more separation of your software
  - Container = "box" where software is in "installed"
  - You tell your PC to use this "box" when you want to run the tool
  - No need to install it on the device: it's installed in the box
  - You *pull* the container, then run the command
- More HPC information: in CalcUA slides

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## Ways to install & use software: flowchart

#### How to download my needed software?

- FIRST: Google the tools you need, inspect the size of your data, ask AI, look for similar publications, compare alternatives, ...
- Inspect the documentation of the tools
  - Popular tools: easily findable on Google, conda, docker (containers) etc.
  - Also often: GitHub pages → look for the README file: this should contain the information to
    install the tool you want to use

#### Determine what device you will use for your analysis

- Does your own PC suffice (enough memory, computational power, ...)?
- Part of it on HPC of completely?



## Ways to install & use software: flowchart





## Manual installation (local)

- Fastp (alternative to FastQC)
  - https://github.com/OpenGene/fastp
  - Options to install:
    - Bioconda
    - Install binaries (?)
  - Manual installation

```
# download the latest build
wget http://opengene.org/fastp/fastp
chmod a+x ./fastp
```



## Manual installation (local)

#### Fastp (alternative to FastQC)

- Installation steps:
- 1. Download the tool (using wget)
- 2. Make the binary executable (meaning you give yourself the permission to run it)
- 3. Move the binary to a directory in your PATH



## Linux environment: special variables

## \$PATH

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- List of locations where Linux search for programs
  - So when using cat, it knows where to search for this
- Is pre-defined but you can add paths to it
  - Locations in the list are separated with ":"
  - Order matters: first looks in first location, then second...

#### You can change your \$PATH variable

- Just as another variable
- Use command export to set the path for the current session (else you overwrite it!)

localadmin@ngscoursevm:/home/training/data\_files\$ echo \$PATH
/usr/local/sbin:/usr/local/bin:/usr/sbin:/usr/bin:/usr/games:/usr/local/games:/snap/bin
localadmin@ngscoursevm:/home/training/data\_files\$ export PATH=\$PATH:/home//training/
localadmin@ngscoursevm:/home/training/data\_files\$ echo \$PATH
/usr/local/sbin:/usr/local/bin:/usr/sbin:/usr/bin:/usr/games:/usr/local/games:/snap/bin:/home//training/
localadmin@ngscoursevm:/home/training/data\_files\$ echo \$PATH
/usr/local/sbin:/usr/local/bin:/usr/sbin:/usr/bin:/bin:/usr/games:/usr/local/games:/snap/bin:/home//training/
localadmin@ngscoursevm:/home/training/data\_files\$ echo \$PATH

## Manual installation (local)

- Fastp (alternative to FastQC)
  - Installation steps:
  - 1. Download the tool (using wget)
  - 2. Make the binary executable (meaning you give yourself the permission to run it)
  - 3. Move the binary to a directory in your PATH

Often included in the PATH:

- /usr/local/bin (preferred for user-installed binaries)
- /usr/bin (for system-wide binaries)

To move \$ sudo mv ./fastp /usr/local/bin/

4. Verify the installation (try to run the cmd)

## **Error handling**

- Unfortunately, running programs is not always smooth
- Something goes wrong: error code
  - Code 0 means no errors occurred
  - 1: general errors, minor problems
  - 2: severe erorrs
  - ... goes up until 255...
- Get an error?
  - Stay calm
  - Read the error: it often indicates a lot
  - Copy it and paste it into Google/AI

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## **Error handling**

(base) elinet@ELinformatica:/usr\$ mkdir test
mkdir: cannot create directory 'test': Permission denied

(base) elinet@ELinformatica:~\$ rm project/ rm: cannot remove 'project/': Is a directory

(base) elinet@ELinformatica:~/project/LeapSEQ\_nf/data\$ cd.. cd..: command not found



## **Error handling**

```
(base) elinet@ELinformatica:~/project/LeapSEQ_nf/data$ fastqc *.pod5
Failed to process subset_PAK100.pod5
uk.ac.babraham.FastQC.Sequence.SequenceFormatException: ID line didn't start with '@'
        at uk.ac.babraham.FastQC.Sequence.FastQFile.readNext(FastQFile.java:158)
        at uk.ac.babraham.FastQC.Sequence.FastQFile.<init>(FastQFile.java:89)
        at uk.ac.babraham.FastQC.Sequence.SequenceFactory.getSequenceFile(SequenceFactory.ja
va:106)
        at uk.ac.babraham.FastQC.Sequence.SequenceFactory.getSequenceFile(SequenceFactory.ja
va:62)
        at uk.ac.babraham.FastQC.Analysis.OfflineRunner.processFile(OfflineRunner.java:159)
        at uk.ac.babraham.FastQC.Analysis.OfflineRunner.<init>(OfflineRunner.java:121)
        at uk.ac.babraham.FastQC.FastQCApplication.main(FastQCApplication.java:316)
Failed to process subset_plasmo100.pod5
uk.ac.babraham.FastQC.Sequence.SequenceFormatException: ID line didn't start with '@'
        at uk.ac.babraham.FastOC.Sequence.FastOFile.readNext(FastOFile.java:158)
        at uk.ac.babraham.FastQC.Sequence.FastQFile.<init>(FastQFile.java:89)
        at uk.ac.babraham.FastQC.Sequence.SequenceFactory.getSequenceFile(SequenceFactory.ja
va:106)
        at uk.ac.babraham.FastQC.Sequence.SequenceFactory.getSequenceFile(SequenceFactory.ja
va:62)
        at uk.ac.babraham.FastQC.Analysis.OfflineRunner.processFile(OfflineRunner.java:159)
        at uk.ac.babraham.FastQC.Analysis.OfflineRunner.<init>(OfflineRunner.java:121)
        at uk.ac.babraham.FastOC.FastOCApplication.main(FastOCApplication.java:316)
```

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# **Exercises part 7**



## **Running software**

- Download fastQC on your system (not with conda) (tip: apt-get)
- Run fastQC in data\_files/fastq, on all the fastq.gz files
  - Direct the output to a subdirectory called fastqc\_output
  - Use the documentation of fastqc to find out how
  - How do you select the .fastq.gz files only?
  - What is your output? Is this useful?
     mkdir fastqc\_output
  - Fastqc –o fastqc\_output/ data\_files/fastq/\*.fastq.gz
- Download multiqc (not with conda)
  - pip install multiqc
- Run multiqc
  - Which input does it need? How do you run it? Why would you use this tool?
  - multiqc fastqc\_output/.



# 8. Bash scripting



## VSCode

- To code/write programs effectively, we make use of code editors
- Enables a nice interface to edit scripts
- VSCode is widely used, convenient code editor



## VSCode

- Check installation VSCode
- Windows users: Connect to WSL
- Open data\_files folder
- Create new folder 'bash\_scripts'



## **Programming in bash**

#### Bash = command processor

- Interprets all the commands you type
- Linux terminal = program to interact with bash
- So all this time, we've been using bash

### Single commands versus ...

- One-liner scripts
  - Using pipes, seen previously
- Bash scripts
  - For more advanced commands: sometime need to iterate a command
    - for loop



## **Programming in bash**

#### Bash script: ends with .sh

\$ test.sl	ı X	
\$ test.s	sh	
1	#!/bin/bash	→ shebang
2		
3	<pre># Script metadata/comments</pre>	After the #: comment. not interpreted
4	# Author: Your Name	
5	# Date: YYYY-MM-DD	
6	# Description: What the script does	
7	# Usage: How to use the script	
8		
9	<pre>cd /home/elinet/linux_course/data_files</pre>	
10	mkdir hello	
11	cd hello	Bunch of commands
12	<pre>echo "This is a message" &gt; message.txt</pre>	
13		

#### Run the script by: \$ bash test.sh



## **Programming in bash**

- for loop
  - Do something (one or more commands) for a list of files/folders/variables
  - Syntax when making a script: for <AN\_ITEM> in <A\_LIST\_OF\_ITEMS> do

#do some stuff with the loop variable \$<AN\_ITEM>

#### done

- To use on command line, separating the different steps with semicolon;
  - In general: semicolon in bash works as a space: both separate commands
  - **for** <AN\_ITEM> **in** <A\_LIST\_OF\_ITEMS>; do ...; **done**

training@ngscoursevm:~\$ for NAME in Tycho Dale Philip; do echo "\$NAME would like to help you learn Linux"; done Tycho would like to help you learn Linux Dale would like to help you learn Linux Philip would like to help you learn Linux training@ngscoursevm:~\$



## **Programming in bash: FOR loop**

- for <AN\_ITEM> in <A\_LIST\_OF\_ITEMS>; do ... ; done
  - <AN\_ITEM> = Loop variable
    - Can be any name. Name it to use in loop
  - <A\_LIST\_OF\_ITEMS>
    - Can be a list of items
      - Tycho Dale Philip
      - Separated with a spaces
    - Can be a command
      - Need to enclose with backticks (`)

training@ngscoursevm:~\$ for FILE in `ls /home/training/data\_files/fastq`; do echo \$FILE; done | head -5
Sample43\_S91\_L001\_I1\_001.fastq
Sample43\_S91\_L001\_I2\_001.fastq
Sample43\_S91\_L001\_I2\_001.fastq.gz
Sample43\_S91\_L001\_R1\_001.fastq
training@ngscoursevm:~\$





## **Programming in bash: FOR loop**

#### Loops or wildcards?

#### Sometimes it's just a choice, previous example

localadmin@ngscoursevm:/home/training/data\_files/fastq\$ ls -1 | head -5 Sample43\_S91\_L001\_I1\_001.fastq Sample43\_S91\_L001\_I1\_001.fastq.gz Sample43\_S91\_L001\_I2\_001.fastq Sample43\_S91\_L001\_I2\_001.fastq.gz Sample43\_S91\_L001\_R1\_001.fastq localadmin@ngscoursevm:/home/training/data\_files/fastq\$

#### More advances commands require loops

localadmin@ngscoursevm:/home/training/data\_files\$ cat sample\_id.txt
002
004
008
005
localadmin@ngscoursevm:/home/training/data\_files\$ for SAMPLE in `cat sample\_id.txt` ; do find ID\_SNP -name "\${SAMPLE}.vcf"; done
ID\_SNP/002.vcf
ID\_SNP/004.vcf
ID\_SNP/008.vcf
ID\_SNP/008.vcf
ID\_SNP/045.vcf
localadmin@ngscoursevm:/home/training/data\_files\$



## **Programming in bash: FOR loop**

#### Loops or wildcards?

#### Sometimes it's just a choice, previous example

localadmin@ngscoursevm:/home/training/data\_files/fastq\$ ls -1 | head -5 Sample43\_S91\_L001\_I1\_001.fastq Sample43\_S91\_L001\_I1\_001.fastq.gz Sample43\_S91\_L001\_I2\_001.fastq Sample43\_S91\_L001\_I2\_001.fastq.gz Sample43\_S91\_L001\_R1\_001.fastq localadmin@ngscoursevm:/home/training/data\_files/fastq\$

#### More advances commands require loops

localadmin@ngscoursevm:/home/training/data\_files\$ cat sample\_id.txt
002
004
008
005
localadmin@ngscoursevm:/home/training/data\_files\$ for SAMPLE in `cat sample\_id.txt` ; do find ID\_SNP -name "\${SAMPLE}.vcf"; done
ID\_SNP/002.vcf
ID\_SNP/004.vcf
ID\_SNP/008.vcf
ID\_SNP/008.vcf
ID\_SNP/045.vcf
localadmin@ngscoursevm:/home/training/data\_files\$



# **Exercises part 8**



#### Exercise 1: introductory

You are given following bash script:

#!/bin/bash

echo "Welcome to Bash scripting!"

```
echo "Hello, ${USER}! Nice to meet you."
```

cd ~

echo "You have reached your destination: \$PWD"

- what does it do? It will print "welkom to bash scripting" in the terminal, then print "Hello, <username>! Nice to meer you. Change the directory to the /home directory and then print again in the termina "You have reached your destination: met het absolute path van de home directory"
- 2. create a file and copy the script in there: \$ nano bash\_script1.sh
- 3. set the correct permissions: \$ chmod +x bash\_script1.sh
- 4. run the script: \$ ./bash\_script1.sh

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#### **Exercise 2: intermediate**

Write a bash script that creates a file with following 1. contents:

the user who created the file,

- the date when the file was created, and
- the directory where the file is created.

Format this information however you like. Give the file a unique name, so that it will not overwrite any of your own file\_name=intro\_linux\_ex7.2.txt files ;)

Run you script 2.

#### Add your current working directory (where your script echo ""Created by: \$user"" > ""\$file\_name"" 3. is located! cd to the directory of your script if echo ""Date created: \$date\_created"" >> ""\$file\_name"" needed!) to the path: \$ export PATH=\$PWD:\$PATHecho ""Directory: \$current\_dir"" >> ""\$file\_name"" cd to another directory (e.g. your Desktop, somewhere where your script is NOT located) now you should be able to run your script by only typing the filename, not its entire filepath (eg: bash\_script2.sh)

#!/bin/bash

# Get the username of the user running the

scriptuser=\$USER

# Get the current working directory

current dir=\$PWD

# Get the current date and time

date created=\$(date)

# set a file name:

# ALTERNATIVE, but more advanced: generate a unique

file name: file name=""info file \$(date +%s).txt""

# Write the information to the file

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#### Exercise 3: advanced

#### What is the meaning of \$1 in following bash script?

#!/bin/bash

- # A script to count the number of characters in a command-line argument using wc
- # Use wc to count the number of characters

```
char_count=$(echo -n "$1" | wc -c)
```

```
# Print the result
```

```
echo "The input '$1' has $char_count characters."
```

This is the first argument that you give while executing your script for example: \$ script argument, then argument will be used in the script

Can you think of some use cases for \$i?

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#### Exercise 4: advanced

#### What does this script do?

```
#!/bin/bash
while echo -n "enter number: "; read NUM
  do
  if [ $NUM -eq $NUM ] 2>/dev/null; then
    •
  else
    echo " $NUM is not a number"
    continue
  fi
  if [[ $(( $NUM % 2 )) -eq 0 ]]; then
    echo " $NUM is an even number"
    continue
  fi
    echo " $NUM is an odd number"
    break
done
```



## **Exercises on FOR loops**

#### Exercise 1

- 1. Write a Bash script that creates 10 empty files named file1.txt, file2.txt, ..., file10.txt in the current directory
- 2. Print a message to the terminal for each file created, confirming its creation.

	File	file1.txt	has	been	created.
Expected output	File	file2.txt	has	been	created.
	File	file3.txt	has	been	created.
	File	file4.txt	has	been	created.
	File	file5.txt	has	been	created.
	File	file6.txt	has	been	created.
	File	file7.txt	has	been	created.
	File	file8.txt	has	been	created.
	File	file9.txt	has	been	created.
	File	file10.txt	t has	s beer	n created



## **Exercises on FOR loops**

#### Exercise 2: Advanced

- 1. Create a directory called project.
- 2. Navigate into the project directory.
- 3. Create 5 files named file1.txt, file2.txt, ..., file5.txt.
- 4. Write the current date and time into each of the 5 files.
- 5. Copy all files with names starting with file into a new subdirectory called backup.
- 6. Rename all files in the backup directory by adding a .bak extension (e.g., file1.txt  $\rightarrow$  file1.txt.bak).
- 7. Delete the original files (file1.txt to file5.txt) from the project directory.
- 8. Print a message indicating that the script completed successfully.

#### With the second second



# Thank you for your attention!