RCBio: easy, quick HPC pipeline builder & runner

HMS Research Computing

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What is RCBio?

- RCBio is a pipeline runner and workflow tool developed and supported by HMS RC consultants.
- It was initially designed for the Lee lab for their complex 15+ step image analysis workflow.
- The project was later expanded to all users on our previous cluster, Orchestra, which is now retired.
- RCBio was ported to our current cluster, O2, and we have continued development of the tool. RCBio currently supports job submission to the Slurm scheduler.

Advantages of using RCBio

- *Proven track record*: The RCBio project has already helped many HMS researchers with great successes.

- *Quick to setup and run*: Researchers can easily build and run new HPC pipelines and share with colleagues and collaborators.

- *Assistance available:* HMS RC consultants also build selected pipelines and share with researchers, as well as provide support for RCBio.

User comments about RCBio

"PS-- **this pipeline runner is THE most amazing thing**. Super organized, super easy to rerun failed jobs, and so easy to debug right from my email because the failure emails are so informative. Thank you for creating this!!"

"I was able to code these steps up in a single bash script with rcbio pipeline decorators; all tracking and job dependencies were taken care of and I received emails for successful and failed jobs. On AWS, the time to code and run these steps would have been significantly longer. First, …. In contrast, error logs generated by rcbio are intuitively named and located, and the pipeline runner enables us to automatically resubmit failed jobs only (all at once, instead of one-by-one). Second, … . Using the pipeline runner, subsequent jobs automatically begin running once their dependencies have been satisfied."

-- Shilpa Kobren, a postdoc from HMS DBMI

How do I access RCBio?

- RCBio is available through the module system on our cluster O2:

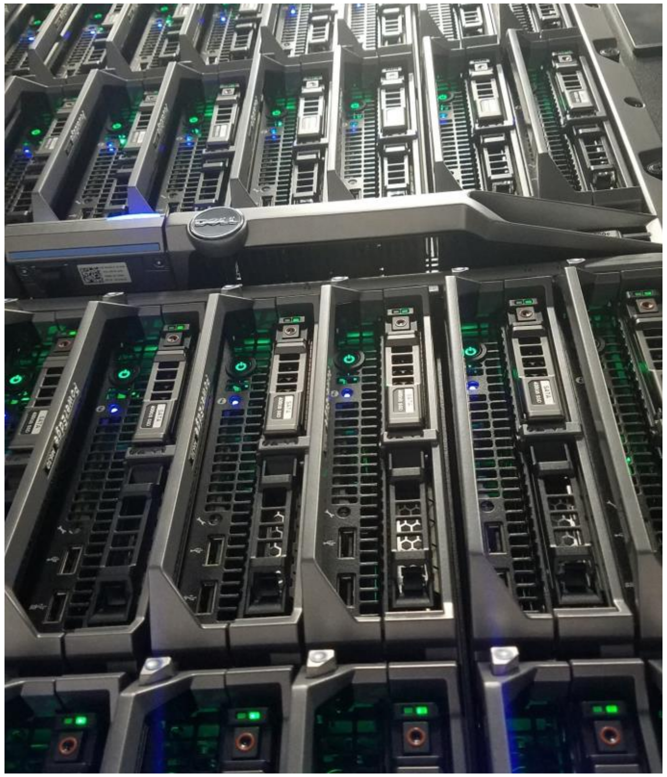
```
# shows currently available versions of rcbio
$ module spider rcbio

# load version 1.3 of rcbio
$ module load rcbio/1.3
```

- This class does require knowledge of O2 and the SLURM scheduler.

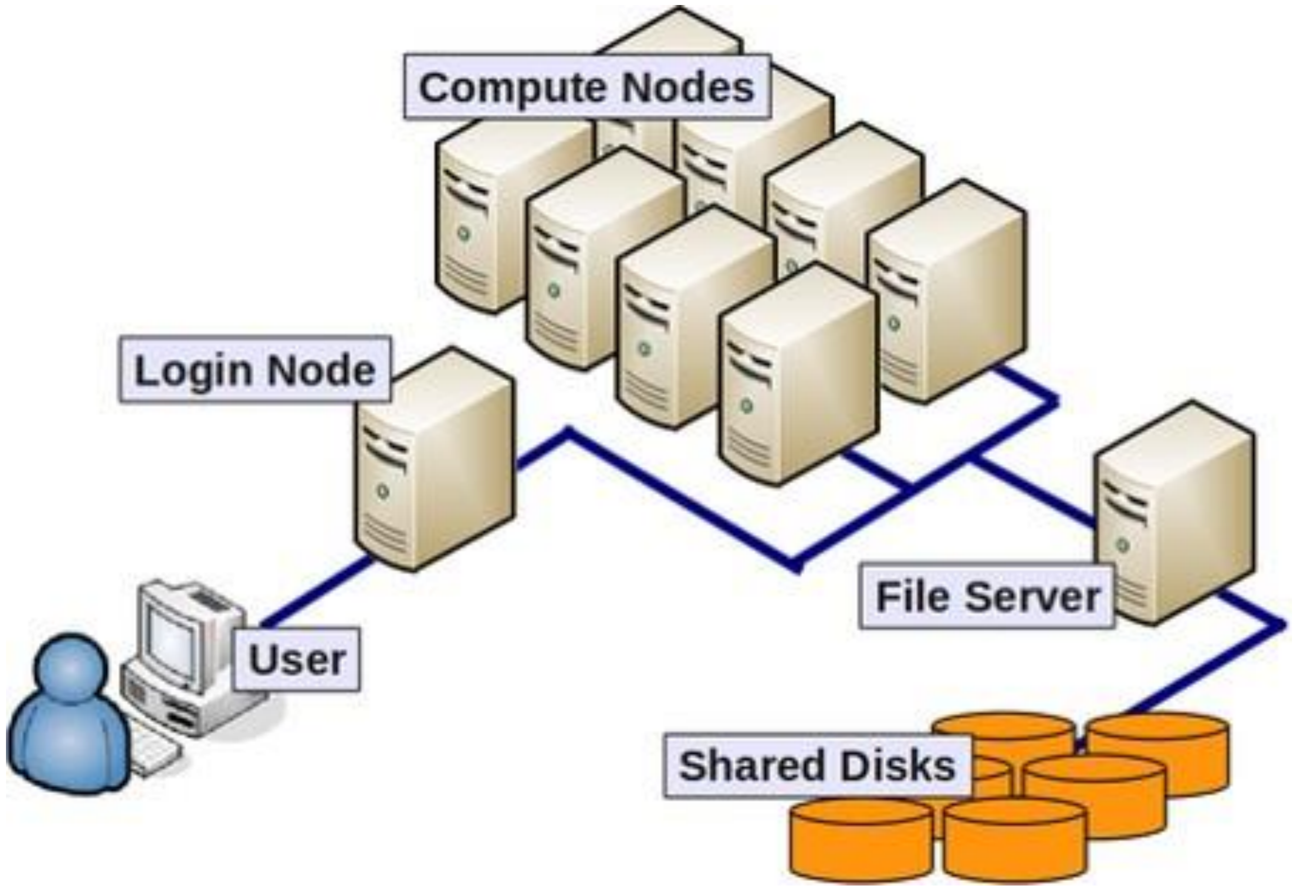
- We will have a quick review of using HPC, such as O2, and the SLURM scheduler now, for those who aren't familiar.

High Performance Computing (HPC)

- Hundreds/thousands of computers with a lot of CPUs and memory
- Dedicated scheduler for parallel processing
- Shared software environment
- Shared storage
- Great support
    - rchelp@hms.harvard.edu



Generic Cluster Architecture



Ways to submit jobs on O2 using the SLURM scheduler

- The login servers are not designed to handle intensive processes, and CPU usage is throttled. We need to submit jobs that request resources (like # cpus, memory, time, etc.) instead!

- Two types of jobs:
  - Interactive jobs using `srun`
    - Programs can be run directly once on compute node

  - Batch jobs using `sbatch`
    - Requires writing a job script (a text file) to submit

Interactive Job example

```
mfk8@login0~$ srun --pty –p interactive –t 0-2:00 --mem 2G bash
```

“`srun --pty`” is how interactive jobs are started

“`-p interactive`” is the partition

“`-t 0-2:00`” is the time limit (2 hours)

“`--mem 2G`” is the memory requested, 2GiB

```
mfk8@compute-a:~$
```

Once on the compute node, you can run any command you want!

```
mfk8@compute-a:~$ bowtie -c 4 hg19 file1_1.fq file1_2.fq
```

Batch job example script

```
#!/bin/bash
#SBATCH -p short #partition
#SBATCH -t 0-01:00 #time days-hr:min
#SBATCH -c X #number of cores
#SBATCH --mem=XG #memory per job (all cores), GiB
#SBATCH -o %j.out #out file
#SBATCH -e %j.err #error file
#SBATCH --mail-type=BEGIN,END,FAIL,ALL
#SBATCH --mail-user=mfk8@med.harvard.edu
# put any module load commands here
# put any analysis commands you want to run here
```

Assuming we saved the script above as myjobscript.sh, submit it with sbatch like so: `sbatch myjobscript.sh`

Partitions (queues): -p

Partition	Priority	Max Runtime	Max Cores	Limits
short	12	12 hours	20	
medium	6	5 days	20	
long	4	30 days	20	
interactive	14	12 hours	20	2 job limit
priority	14	30 days	20	2 job limit
mpi	12	5 days	640	20 core min
highmem		5 days	20	
gpu, gpu_quad, gpu_requeue		160 GPU hours	34 (total)	420GiB (total)
transfer		5 days	4	

Wall-Time: -t

- `-t days-hours:minutes`
- `-t hours:minutes:seconds`
- Need to specify how long you estimate your job will run for
- Aim for 125% over
- Subject to maximum per partition
- Excessive wall-time (like partition max) takes longer to dispatch, and affect fair-share

CPU: -c

- `-c X` to designate CPU: max 20
- `-N X` to constrain all cores to X nodes
- CPU time: wall time (-t) * (-c) CPUs used
- Unable to use CPU not requested (no overefficient jobs): cgroups constraint
- Adding more cores does not mean jobs will scale linearly with time, and causes longer pend times

Memory: --mem

- Only 1GiB is allocated by default
- `--mem XG` #total memory over all cores
- `--mem-per-cpu XG` #total memory per CPU requested, use for MPI
- If you don't include a unit request (like GiB), it defaults to Mebibytes

Output/Error Files

- Can add jobid to filename with `%j`
- Sample:
  ```
  -e %j.err
  -o %j.out
  ```
- SLURM by default creates this outfile: `slurm-<jobid>.out`
- Additional Flags
- %a job array id
- %A master array job id
- %N node name
- %u user id

Mail

- Mail is not auto-generated upon completion/failure
- `#SBATCH --mail-type= NONE, BEGIN, END, FAIL, REQUEUE, ALL`
- `#SBATCH --mail-user=mfk8@med.harvard.edu`
- Not recommended, not a verbose output
- Use `O2sacct` or `sacct` commands instead

Job Dependencies

- `sbatch -d (or --dependency=)`
- `after:jobid` #(asynchronous)
- `afterany:jobid` #after exit or done
- `afterok:jobid` #success, exit code 0
- `afternotok:jobid` #failure
- `singleton` #after jobs with same name have terminated
- `--kill-on-invalid-dep=<yes|no>` #kill on unmet dependency (on by default)

Job Monitoring: Current jobs

- `$ O2squeue`
  - JOBID, PARTITION, STATE, TIME_LIMIT, TIME, NODELIST(REASON), ELIGIBLE_TIME, START_TIME, TRES
  - [O2squeue documentation]

- *Other options*:
- `$ squeue -u eCommons –t RUNNING/PENDING`
- `$ squeue -u eCommons –p Partition`
- `$ squeue -u eCommons –start`

- *Detailed job info:*

```
$ scontrol show jobid <jobid>
```

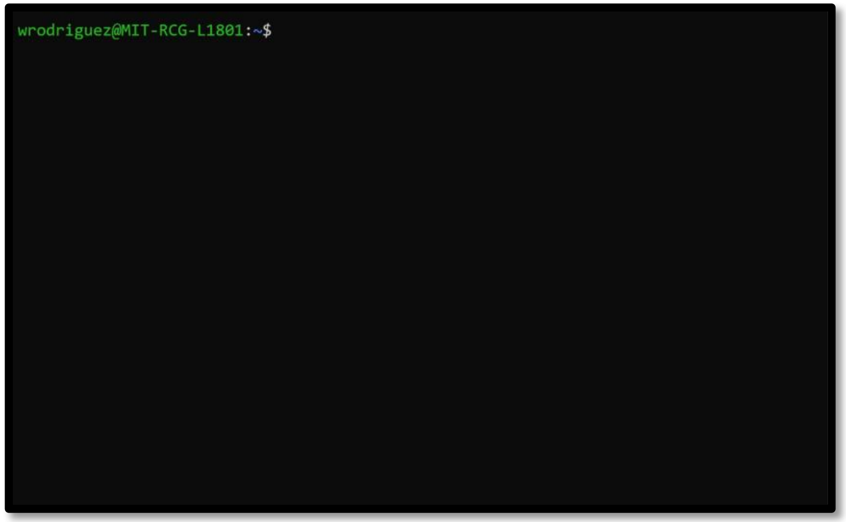
Job Information: Past Jobs

- `$ O2sacct`
    - JobID, Partition, State , NodeList, Start, Timelimit, Elapsed, CPUTime , TotalCPU, AllocTRES, MaxRSS
    - Can specify job ID, job status, and/or timeframe to report accounting info for
    - [`O2sacct documentation`]

- *Other options*:
- `$ sacct -j jobid`
- `$ sacct -r partition`
- `$ squeue -s state`
- `$ sacct --helpformat` #get available fields you can specify

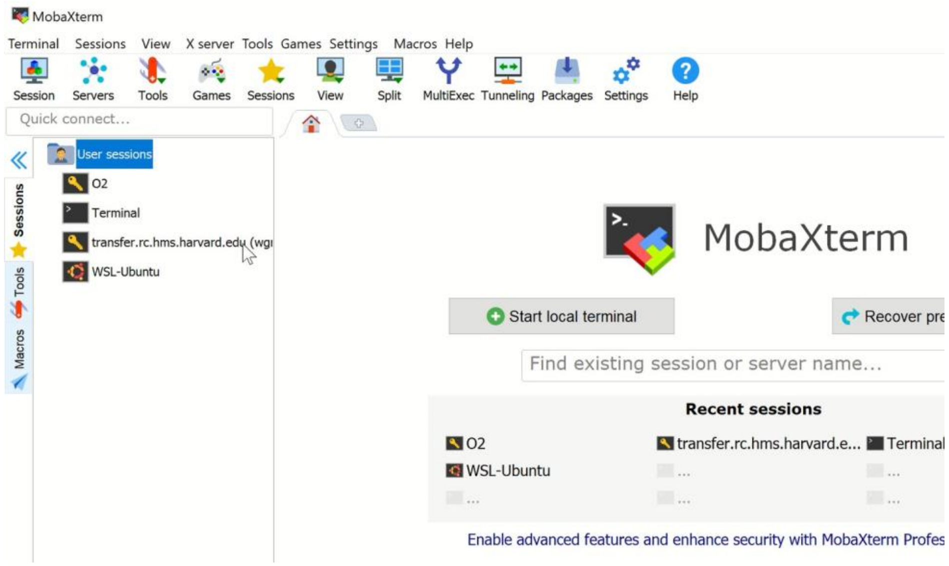
Connecting to O2…

MAC/Linux: Terminal



```
wrodriguez@MIT-RCG-L1801:~$
```

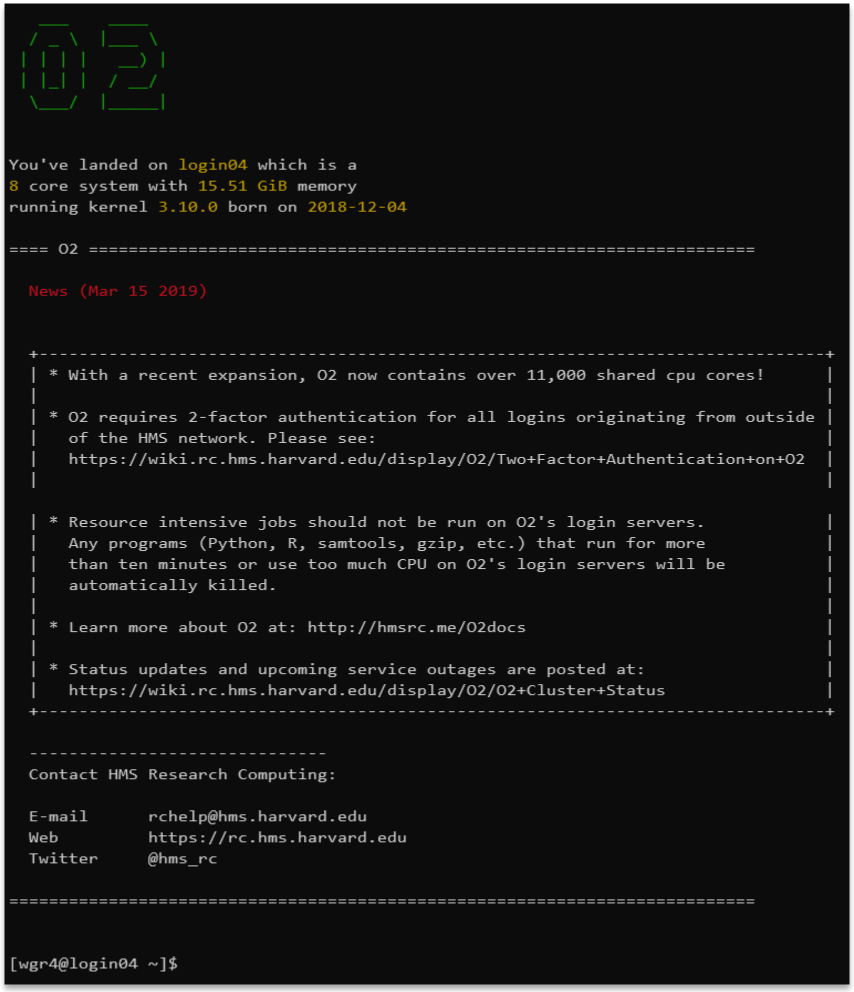
Windows: MobaXterm


MobaXterm
Terminal Sessions View X server Tools Games Settings Macros Help
Session Servers Tools Games Sessions View Split MultiExec Tunneling Packages Settings Help
Quick connect...
User sessions
O2
Terminal
transfer.rc.hms.harvard.edu (wgı
WSL-Ubuntu
Sessions
Tools
Macros
MobaXterm
Start local terminal
Recover pre
Find existing session or server name...
Recent sessions
O2
transfer.rc.hms.harvard.e...
Terminal
WSL-Ubuntu
Enable advanced features and enhance security with MobaXterm Profes

```
ssh yourhmsid@o2.hms.harvard.edu
```

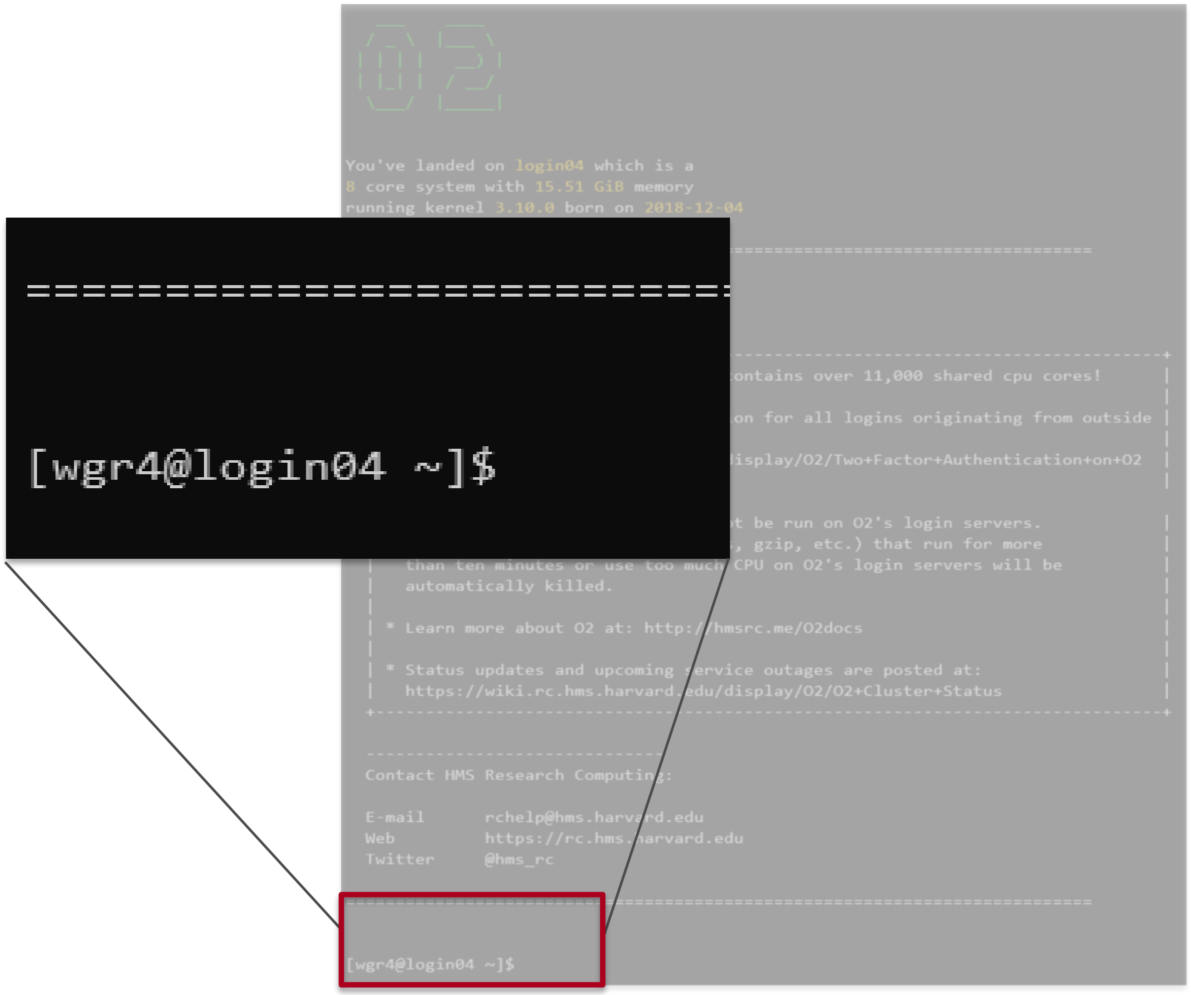
2-Factor (when necessary): Choose 1/2/3 (push/phone/sms)

Welcome to O2!



```
   ___    ____
  / _ \  |___ \
 | | | |   __) |
 | |_| |  / __/
  \___/  |_____|


You've landed on login04 which is a
8 core system with 15.51 GiB memory
running kernel 3.10.0 born on 2018-12-04

==== O2 ===================================================================

  News (Mar 15 2019)


  +--------------------------------------------------------------------------+
  | * With a recent expansion, O2 now contains over 11,000 shared cpu cores! |
  |                                                                          |
  | * O2 requires 2-factor authentication for all logins originating from outside |
  |   of the HMS network. Please see:                                        |
  |   https://wiki.rc.hms.harvard.edu/display/O2/Two+Factor+Authentication+on+O2 |
  |                                                                          |

  | * Resource intensive jobs should not be run on O2's login servers.       |
  |   Any programs (Python, R, samtools, gzip, etc.) that run for more       |
  |   than ten minutes or use too much CPU on O2's login servers will be     |
  |   automatically killed.                                                  |
  |                                                                          |
  | * Learn more about O2 at: http://hmsrc.me/O2docs                         |
  |                                                                          |
  | * Status updates and upcoming service outages are posted at:             |
  |   https://wiki.rc.hms.harvard.edu/display/O2/O2+Cluster+Status           |
  +--------------------------------------------------------------------------+

  ------------------------------
  Contact HMS Research Computing:

  E-mail      rchelp@hms.harvard.edu
  Web         https://rc.hms.harvard.edu
  Twitter     @hms_rc

===========================================================================


[wgr4@login04 ~]$
```

Welcome to O2!



```
You've landed on login04 which is a
8 core system with 15.51 GiB memory
running kernel 3.10.0 born on 2018-12-04

=================================================================


                         -------------------------------------------------------+
                         contains over 11,000 shared cpu cores!                 |
                                                                                |
                         on for all logins originating from outside             |
                                                                                |
                         display/O2/Two+Factor+Authentication+on+O2             |
                                                                                |

                         t be run on O2's login servers.                        |
                         , gzip, etc.) that run for more                        |
 |   than ten minutes or use too much CPU on O2's login servers will be        |
 |   automatically killed.                                                      |
 |                                                                              |
 | * Learn more about O2 at: http://hmsrc.me/O2docs                             |
 |                                                                              |
 | * Status updates and upcoming service outages are posted at:                 |
 |   https://wiki.rc.hms.harvard.edu/display/O2/O2+Cluster+Status               |
 +------------------------------------------------------------------------------+


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 Contact HMS Research Computing:

 E-mail      rchelp@hms.harvard.edu
 Web         https://rc.hms.harvard.edu
 Twitter     @hms_rc

=================================================================


[wgr4@login04 ~]$
```
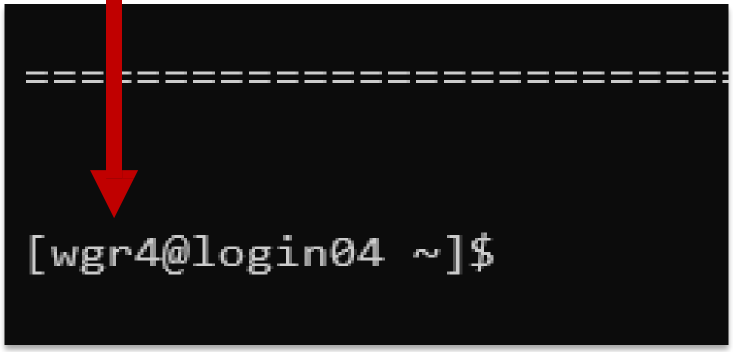

```
===============================


[wgr4@login04 ~]$
```

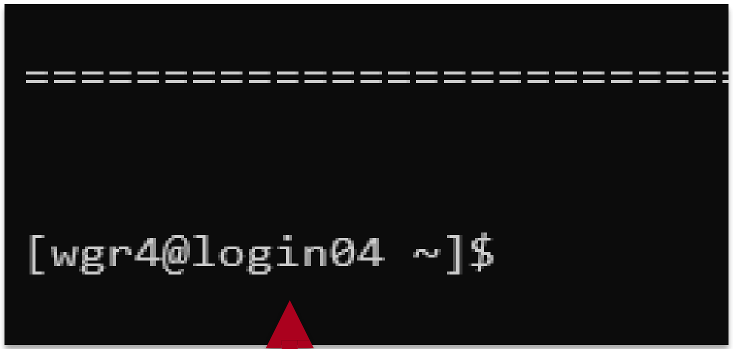
Welcome to O2!

Your HMS ID



```
==============================

[wgr4@login04 ~]$
```

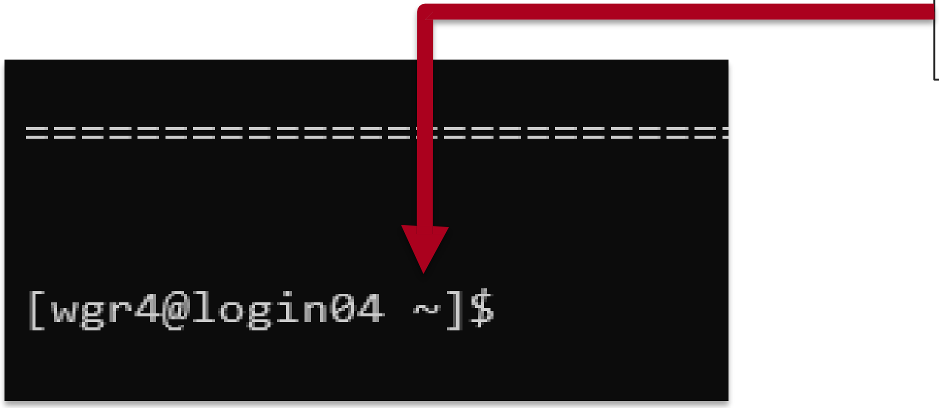
Welcome to O2!



```
==============================

[wgr4@login04 ~]$
```

You are logged into a "shell login server"
Do **not** meant for heavy lifting!

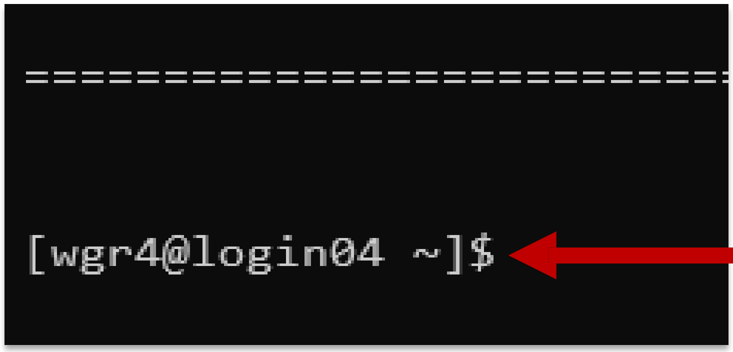
Welcome to O2!



```
==============================

[wgr4@login04 ~]$
```

You’re in your HOME directory.
/home/<HMSID>

Welcome to O2!



```
================================

[wgr4@login04 ~]$
```

Ready to receive commands!

Bash commands

- cd: go to a directory
- ls: list directory and file
- cp: copy directory and file
- mkdir: create new directory
- less: view file content
- cat: show file content

Automate commands with a “for” loop

- Repeat commands against an designated list
  - this syntax is for bash, but other shells (tcsh) are different

- Examples

```
$ for i in 1 2 3 ; do mkdir $i ; done
$ for i in `cat list.txt` ; do cp $i ~/work ; done
```

Bash variable

Variable name must begin with alphabet character or underscore character (_), followed by one or more alphanumeric or underscore characters.

- Assign value:

varName=“value”

varName=value

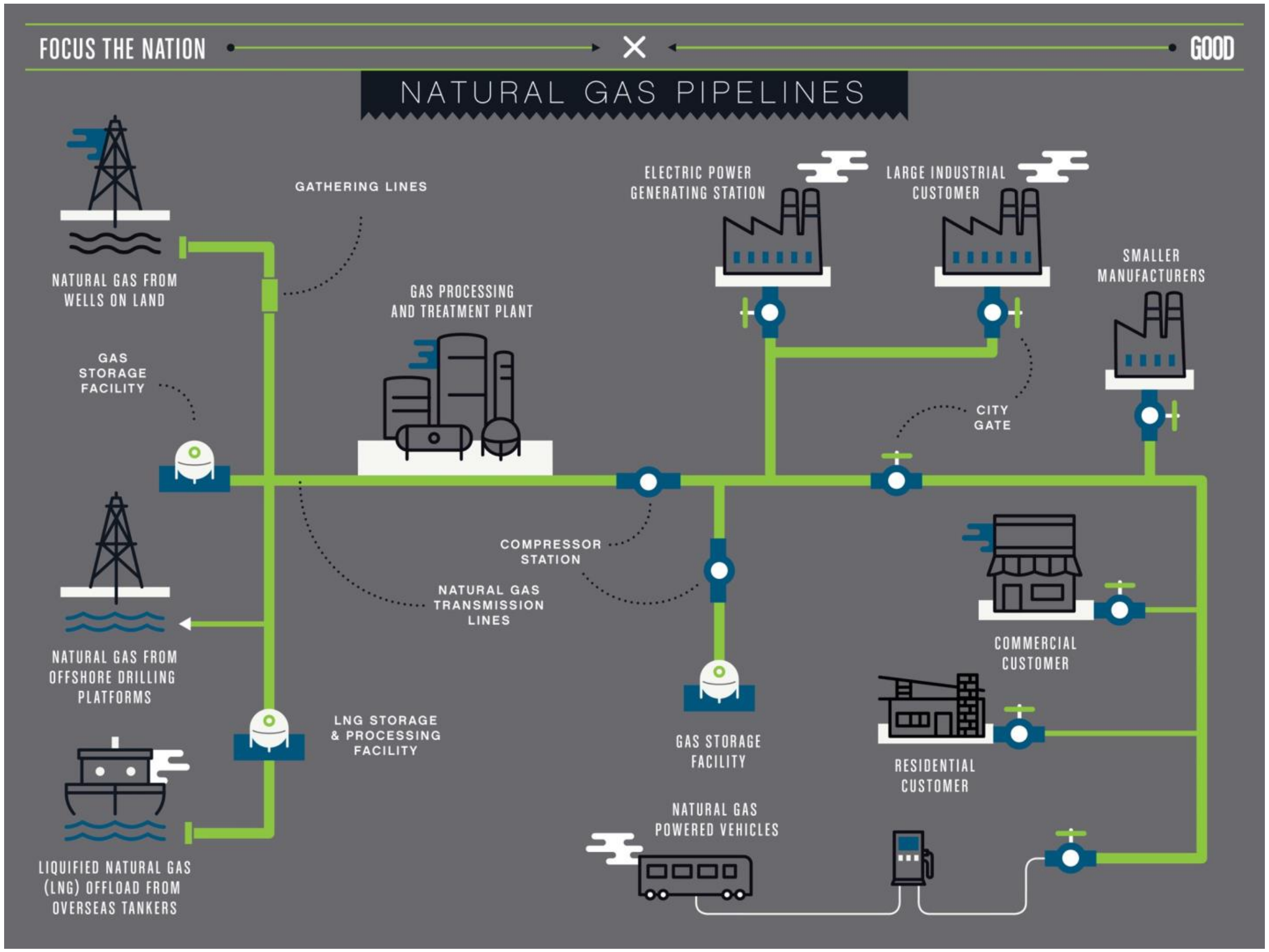
- Refer variable:

${varName}, $varName, “$varName”, “$varName“

Part of Natural gas pipeline

-- https://en.mercopress.com/data/cache/noticias/27951/0x0/gas.jpg

The whole picture


FOCUS THE NATION
GOOD
NATURAL GAS PIPELINES
GATHERING LINES
NATURAL GAS FROM WELLS ON LAND
GAS STORAGE FACILITY
GAS PROCESSING AND TREATMENT PLANT
ELECTRIC POWER GENERATING STATION
LARGE INDUSTRIAL CUSTOMER
SMALLER MANUFACTURERS
CITY GATE
COMPRESSOR STATION
NATURAL GAS TRANSMISSION LINES
NATURAL GAS FROM OFFSHORE DRILLING PLATFORMS
COMMERCIAL CUSTOMER
LNG STORAGE & PROCESSING FACILITY
GAS STORAGE FACILITY
RESIDENTIAL CUSTOMER
NATURAL GAS POWERED VEHICLES
LIQUIFIED NATURAL GAS (LNG) OFFLOAD FROM OVERSEAS TANKERS

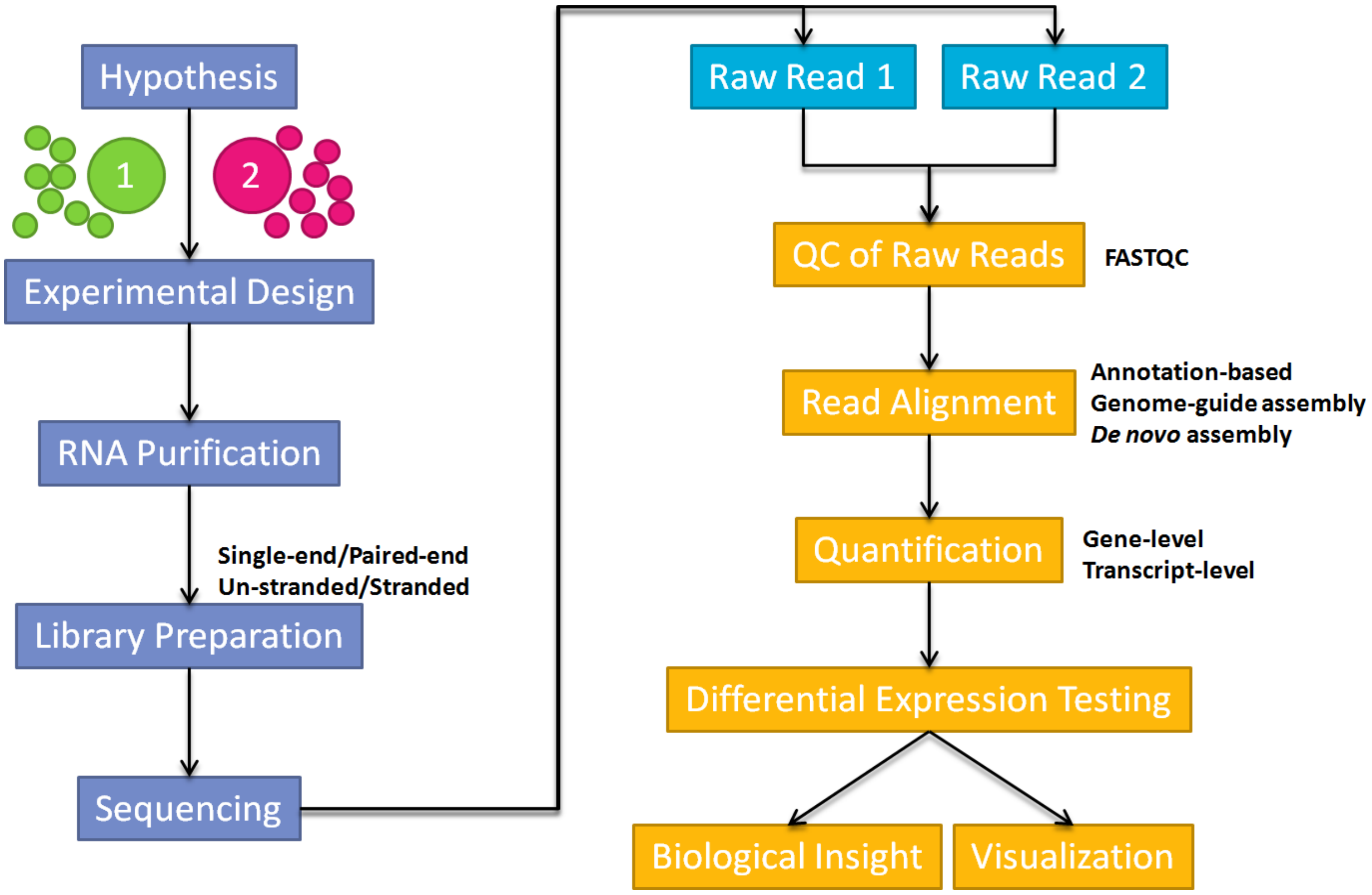
-- https://assets.rbl.ms/19920757/origin.jpg

Data Pipeline

"In computing, a **pipeline**, also known as a **data pipeline**, is a set of data processing elements connected in series, where the output of one element is the input of the next one".

-- https://en.wikipedia.org/wiki/Pipeline_(computing)

RNA-Seq Pipeline



-- https://databeauty.com/figures/2016-09-13-RNA-seq-analysis/rna_seq_workflow.png

Bash path/file name manipulation

```
fileWithPath=/home/id/data/tumor.fq
dirname $fileWithPath            # /home/id/data
basename $fileWithPath           # tumor.fq
basename $fileWithPath .fq   #tumor
```

Bash string manipulation

```
someString=abcdefghijk
echo ${someString#abc}          #defghijk
echo ${someString%ijk}          #abcdefgh

prefix=abc; suffix=ijk
echo ${someString#$prefix}     #defghijk
echo ${someString%$suffix}     #abcdefgh
```

Bash string concatenate

```
sample=tumor; rep=2
echo $sample.$ref                    #  rumor2
echo $sample_$rep                    # does not work
echo /data/group/$sample/$tumor/$rep
```

Catch the output of command

```
output=`cmd -in $input -out $out`

output=$(cmd -in $input -out $out)

output=$(echo today is $(date))

output=`echo today is \`date\``
```

Combine multiple commands

```
cmd1; cmd2; cmd3;

cmd1 && cmd2 && cmd3

mkdir outputDir && cmdToCreateOutput

ls $input && cmd –in $input || exit
```

Creating a Simple Text File

"Nano," "vi", "emacs" are simple command-line editors available.
To create a new file, type the editor you want, then the name of the new file. To edit an existing file, do the same.

```
  mfk8@compute-a:~$ nano myfile.txt
       This is my new file text.
       (Control-X to save (yes) and exit.)
  mfk8@compute-a:~$
  mfk8@compute-a:~$ ls
       myfile.txt
```

Continue RNA-Seq pipeline

Assume we have RNA-Seq data files:

```
ld32@login02 /home/ld32/myRNASeq$ ls
sample1_1.fq  sample1_2.fq  sample2_1.fq
sample2_2.fq  sample3_1.fq  sample3_2.fq
sample4_1.fq  sample4_2.fq
```

RNA-Seq pipeline (single computer)

```
#!/bin/bash
for i in 1 2 3 4; do
      sp=sample${1}; r1=${sp}_1.fq; r2=${sp}_2.fq;

      qcCmd  -input1 ${r1}  -input2 ${r2} –output ${sp}.qc.txt

      alignCmd  -cpu 4 –input1 ${r1}  -input2 ${r2} -output   ${sp}.bam

      countCmd    -input    ${sp}.bam     -output      ${sp}.cnt
done

mergeCmd     -input     *.cnt     -output       allCount.txt

statCmd       -input       allCount.txt       -output       finalResult.txt
```

A simpler pipeline

```
for u in universityA.txt ; do

    grep -H John $u >> John.txt

    grep -H Nick $u >> Nick.txt

done

cat John.txt Nike.txt > all.txt
```

Converting to Slurm pipeline

```
for u in universityA.txt; do
    job1ID=$(sbatch -p short -t 5 --wrap “grep -H John $u >> John.txt”)
    job2ID=$(sbatch -p short -t 5 --wrap “grep -H Nick $u >> Nick.txt”)
done

sbatch -p short -t 5 -d afterok:$job1ID:$job2ID --wrap “cat \
    John.txt Nike.txt > all.txt”
```

Also keep log and send emails

```
job1ID=$(checkIfItIsDone.sh ||
sbatch -p short -t 5 --mem 2G --
wrap “someCmd && touch
job1.success; sendBetterEmail.sh;”)
```

What are checkIfItIsDone.sh? And sendBetterEmail.sh?

A little more complex

```
for i in A    B    C    E    F;   do
    u=university${i}.txt
    grep -H John $u >> John.txt
    grep -H Nick $u >> Nick.txt
done
cat John.txt Nike.txt > all.txt
```

Can we automate it?

```
for i in A    B    C    E    F ; do
    u=university${i}.txt

    #@ Please submit this command as slurm job with 1G memory,
    #@ 5 minute run time and 1 CPU. And this job can start right away.
    grep -H John $u >> John.txt

    #@ Please submit this command as slurm job with 1G memory,
    #@ 5 minute run time and 1 CPU. And this job can start right away
    grep -H Nick $u >> Nick.txt
Done

#@ Please submit this command as slurm job with 2G memory,
#@ 5 minute run time and 1 CPU. And this job needs to wait for the other 10 jobs
cat John.txt Nike.txt > all.txt
```

RCBio decorator

```
#@3,1.2,merge,,sbatch -p short -t 5 –c 1 --mem 1G
cat John.txt Nick.txt >> all.txt
```

#@	→	This row is decorator for RCBio to parse
3	→	Step ID.
1.2	→	The step IDs this step depends on.
merge	→	Step name.
sbatch…	→	sbatch command to use

Can we automate it?

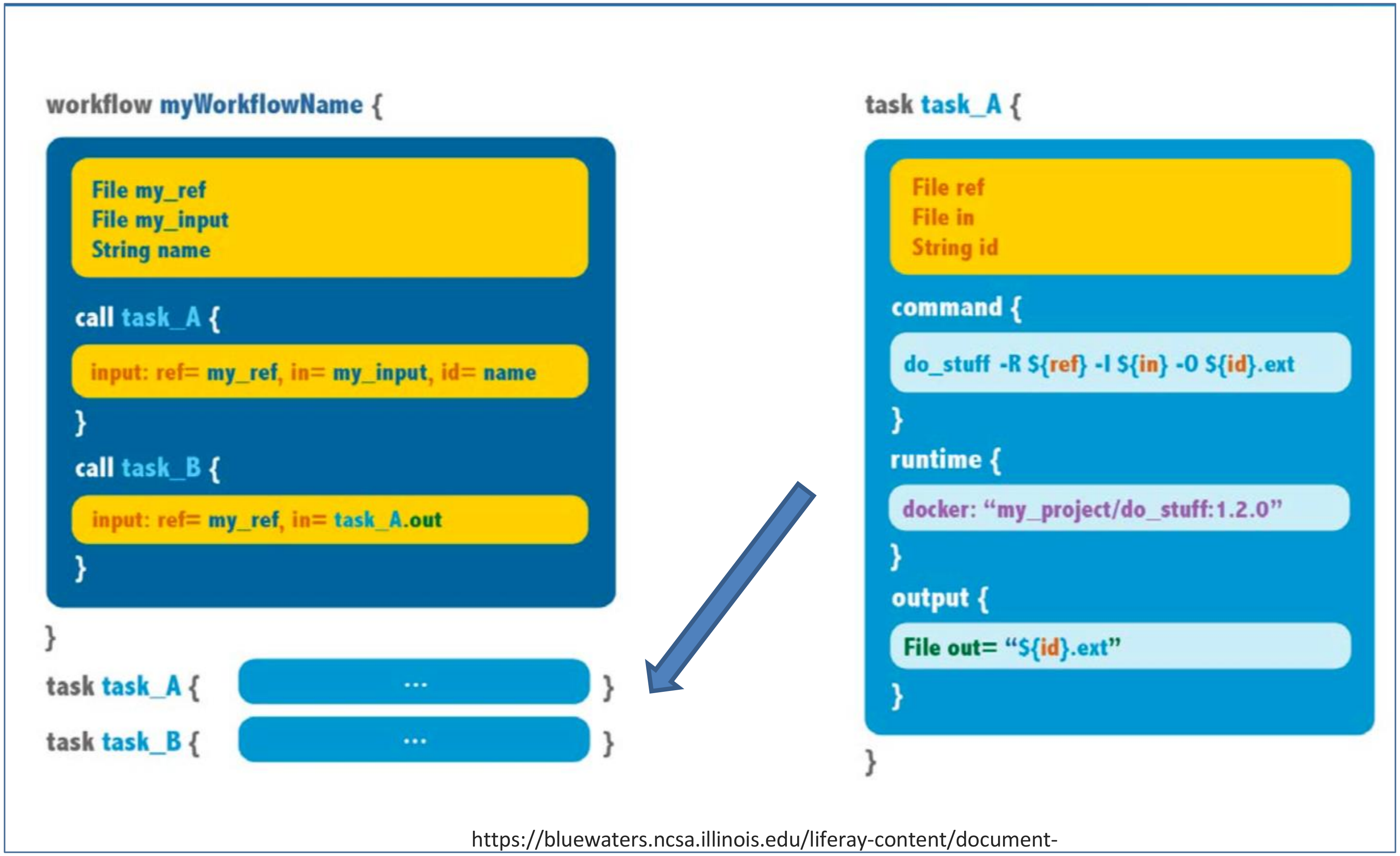
```
for i in A     B     C     E     F ; do
      u=university${i}.txt

      #@1,0,findJohn,u,sbatch -p short -t 5 –c 1 --mem 1G
      grep -H John $u >> John.txt

      #@2,0,findNick,u,sbatch -p short -t 5 –c 1 --mem 1G
      grep -H Nick $u >> Nick.txt
Done

#@3,1.2,merge,,sbatch -p short -t 5 –c 1 --mem 1G
cat John.txt Nike.txt > all.txt
```

Practice session

Go through the wiki page and run a simple pipeline

https://harvardmed.atlassian.net/wiki/spaces/O2/pages/1600651373/RC+workflows

Other solution 1: Cromwell using ‘CWL’ file

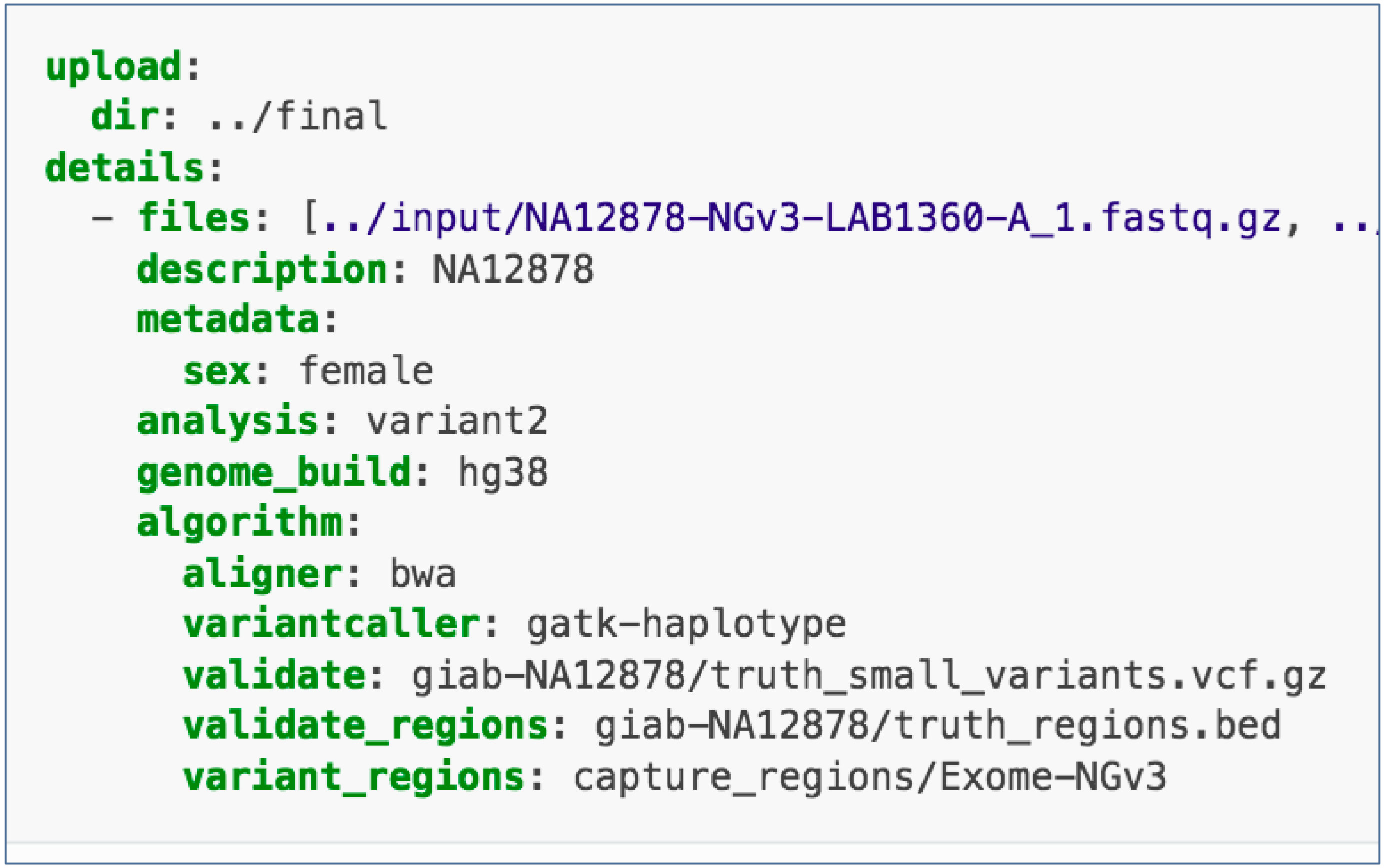


```
workflow myWorkflowName {
    File my_ref
    File my_input
    String name

    call task_A {
        input: ref= my_ref, in= my_input, id= name
    }
    call task_B {
        input: ref= my_ref, in= task_A.out
    }
}
task task_A { ... }
task task_B { ... }
```

```
task task_A {
    File ref
    File in
    String id

    command {
        do_stuff -R ${ref} -I ${in} -O ${id}.ext
    }
    runtime {
        docker: “my_project/do_stuff:1.2.0”
    }
    output {
        File out= “${id}.ext”
    }
}
```

https://bluewaters.ncsa.illinois.edu/liferay-content/document-library/content/Ruchi%20Munshi.%20Blue%20Waters%20webinar.%20Cromwell%20and%20WDL.pdf

Other solution 2: BCBio using ‘yaml’ and ‘CWL’ file



```
upload:
  dir: ../final
details:
  - files: [../input/NA12878-NGv3-LAB1360-A_1.fastq.gz, ..
    description: NA12878
    metadata:
      sex: female
    analysis: variant2
    genome_build: hg38
    algorithm:
      aligner: bwa
      variantcaller: gatk-haplotype
      validate: giab-NA12878/truth_small_variants.vcf.gz
      validate_regions: giab-NA12878/truth_regions.bed
      variant_regions: capture_regions/Exome-NGv3
```

```
bcbio_nextgen.py config/NA12878-exome-methodcmp.yaml -n 8
```

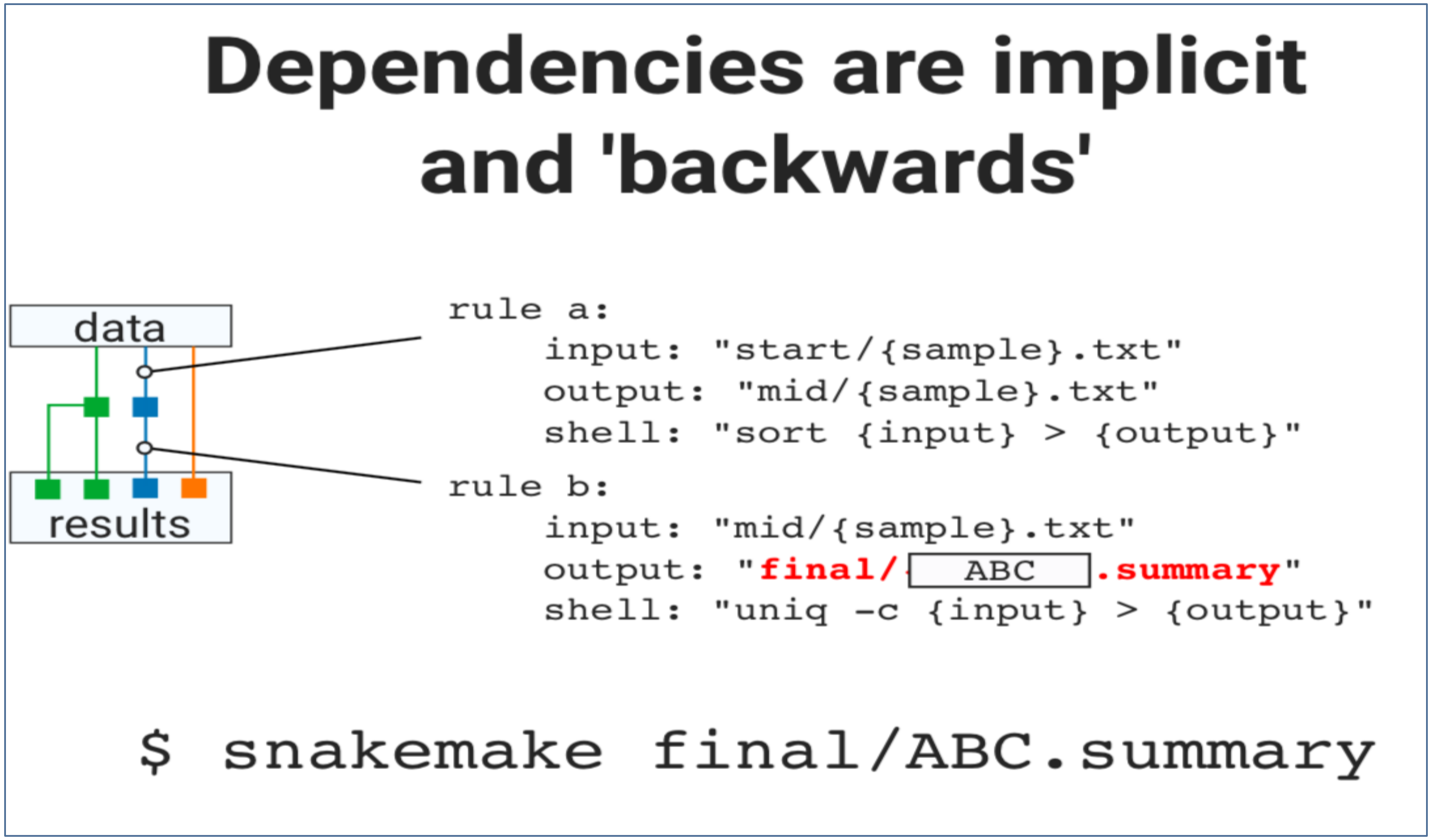
https://bcbio-nextgen.readthedocs.io/en/latest/contents/intro.html#workflow

Other solution 3: NextFlow script

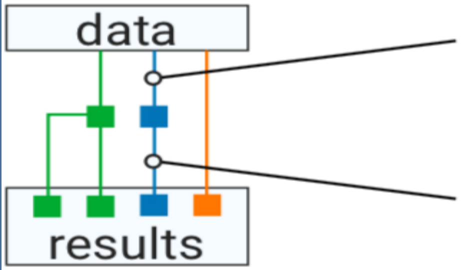
```
#!/usr/bin/env nextflow

params.in = "$baseDir/data/sample.fa"

/*
 * split a fasta file in multiple files
 */
process splitSequences {

    input:
    path 'input.fa' from params.in

    output:
    path 'seq_*' into records

    """
    awk '/^>/{f="seq_"++d} {print > f}' < input.fa
    """
}
```

https://www.nextflow.io/example1.html

Other solution 4: Snakemake using makefile



Dependencies are implicit and 'backwards'



```
rule a:
    input: "start/{sample}.txt"
    output: "mid/{sample}.txt"
    shell: "sort {input} > {output}"
rule b:
    input: "mid/{sample}.txt"
    output: "final/{ ABC }.summary"
    shell: "uniq -c {input} > {output}"
```

```
$ snakemake final/ABC.summary
```

https://hpc.nih.gov/training/handouts/180221_snakemake_class_web.pdf

For more direction

- **Email:** rchelp@hms.harvard.edu
- **O2 wiki**: https://harvardmed.atlassian.net/wiki/spaces/O2/overview
- **Website**: http://rc.hms.harvard.edu
- **RC Office Hours**: Wed 1-3p Gordon Hall 500
  - https://rc.hms.harvard.edu/office-hours/ for Zoom web conferencing during remote work

Please fill out the survey

- Accessible through the Harvard Training Portal
  - https://trainingportal.harvard.edu/
- Click on “Me” then “RCBio: Easy and Quick HPC Pipeline Builder and Runner”
- Scroll to “Evaluations” and click on the survey

- We appreciate any feedback or comments!