Tutorial

PIPEMB-WDL

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1. Preparing the input sequence files:

The input file is formatted as TSV, which is a **simple text format for storing data in** a tabular structure. It is formed by the following columns:

1. Read group identifier
2. Sequence file address (.ubam, .fastq extensions)
3. Sequence file address 2 (for pair-end .fastq files)
4. Line identifier (unique for each line)
5. Sample name, same for all lines of the same sample
6. Sequencing platform
7. Library preparation
8. Pairing: "paired"
9. Sample type: "tumor" or "normal"
10. Paired sample: if you are going to do a tumor/normal somatic study, in this column put the name of the paired sample

Each line represents a reads group.

An example of a multi-sample and multi-read group is given in the next Figure.

group1	/../data/a_R1.fastq	/.../data/a_R2.fastq	1	SAMPLE1	ILLUMINA	lib01	paired
group2	/../data/b_R1.fastq	/../data/b_R2.fastq	2	SAMPLE1	ILLUMINA	lib01	paired
group1	/../data/c_R1.fastq	/../data/c_R2.fastq	3	SAMPLE2	ILLUMINA	lib01	paired
group2	/../data/d_R1.fastq	/../data/d_R2.fastq	4	SAMPLE2	ILLUMINA	lib01	paired

2. Preparing the JSON file.

- Common options and name convention for template configurations:

The JSON files determines what will be executed in the workflow. To recognize what steps are set, a convention for the name of file is followed. For example, we have: pr1g1_SM_Fl_jn0s0pn0f1v1.json, what that means? The nomenclature used is:

- 0: disabled , 1: enabled
- pr: pre-processing
- g: germline
- SM: single sample mode
- MM: multisample mode
- Fl: filtering
- jn: joint genotyping
- s: somatic
- pn: create panel of normal
- f: filter with Funcotator
- v: filter with VEP
- ++/pp: filter with Funcotator with optional MAF and VCF for independent samples

3. Execution:

The script to call the pipeline is in the

/data04/tools/PIPEMB/homologacao/PIPEMB-WDL/DEV/exec/run_workflow_PIPEMB-WDL.slurm

To execute the pipeline is necessary to launch it as a SLURM job using the sbatch command. Also it is necessary to pass the arguments to the pipeline (json file)

An example:

```
sbatch ../../DEV/exec/run_workflow_PIPEMB-WDL.slurm
/data04/tools/PIPEMB/homologacao/PIPEMB-
WDL/TUTORIAL/configs/pr1g1_SM_F1_jn0s0pn0v1f1/001_1_workflow_INCA_qa1pr1g1_SM
_Fl1_jn0s0pn0v1f1pp_.json
```

The pronts shows the name of job summited:

```
Submitted batch job 34908
```

A log of the workflow is created in the execution directory with the name of "workflow-####.log", where the number corresponds to the job number.

4. Output directory

The structure of the results in the selected directory (- output_dir parameter) is the following:

- ***readQualitycontrol/fastqc***: contains the output of the fastqc quality analysis.
- ***bam***: contains bam files resulting from preprocessing. Optional output, if it is used (do_preprocessing = true, default).
- ***<germline/somatic>_vcfs***: contains the vcf files resulting from germline/somatic variants call (HaplotypeCaller + CNNScoreVariants + FIlterVariantTranches / Program Mutect2 + FilterMutectCalls). Note: In the case of somatic, the filename has a T in front of it, but the truth is a vcf with the result for normal and tumor sample from the same sample. Optional output, if it is used (do_<germline/somatic>_short_variant_discovering = true) .
- ***<germline/somatic>_PASS***: filtered vcf, contains only those variants that have "PASS" in the filter column. Optional output, if variant call or annotation is set.
- ***<germline/somatic>_vcfs_merged***: Contains the file final_vcf_all_samples.vcf. It is a multisample vcf, containg the variants present in the germline/somatic_PASS folder in a single file. Optional output, if variant call or annotation is set, and it is a multisample study.
- ***<germline/somatic>_funcotator_annot*:** Contains the file final_vcf_all_samples annotated by Funcotator. Optional output, if it is used (germline/somatic_annot_with_funcotator = true)
- ***<germline/somatic>_funcotator_indep_samples_annot***: Contains a one file for each sample annotated by Funcotator using VCF format. Optional output, if it is used (germline/somatic_annot_with_funcotator_add_allsamples = true)

- ***<germline/somatic>_funcotator_maf_annot***: Contains a one file for each sample annotated by Funcotator using MAF format. Optional output, if it is used (germline/somatic_annot_with_funcotator_add_maf = true)
- ***<germline/somatic>_vep_annot***: Contains file final_vcf_all_samples annotated by Funcotator (if defined) and by VEP. Final file resulting from the workflow. Optional output, if it is used (germline/somatic_annot_with_vep = true)

5. File system structure used for examples

To illustrate an example of execution, was created an file structured composed by four directories located in /data04/tools/PIPEMB/homologacao/PIPEMB-WDL/TUTORIAL

- *configs* contains generic JSON files with example of main common options for workflows.
- *data:* contains the data used in the tutorial
- *execution:* directory from which the workflow must be executed for this tutorial. Contains the output log, generated for each execution and the Cromwell directory used during the execution.
- *result:* directory used to copy final outputs.

5.1. Execution of germline short variant call, with preprocessing and annotation.

Data:

2 samples, NA12878_20k, WGS Fastq format, from GATK test data.

Data source link: https://console.cloud.google.com/storage/browser/gatk-test-data/wgs_fastq/NA12878_20k;tab=objects?organizationId=548622027621&project=broad-dsde-outreach&prefix=&forceOnObjectsSortingFiltering=false

Script used to download the data: /data04/tools/PIPEMB/homologacao/PIPEMB-WDL/TUTORIAL/data/gatk-test-data/wgs-fastq/NA12878_20k/getting_data_tutorial.sh

The two samples will be used to execute the short variant call. One sample is composed by two read group and other by one. Each read group is par-end data. The sequence input file used is: /data04/tools/PIPEMB/homologacao/PIPEMB-WDL/TUTORIAL/data/gatk-test-data/wgs-fastq/NA12878_20k/ NA12878_20k_input_data.tsv

Configuration:

The configuration file used is:

/data04/tools/PIPEMB/homologacao/PIPEMB-WDL/TUTORIAL/configs/pr1g1_SM_F1_jn0s0pn0v1f1/001_workflow_INCA_pr1g1_SM_Fl1_jn0s0pn0v1f1pp_.json

Execution:

i. Go to the execution directory

```
cd /data04/tools/PIPEMB/homologacao/PIPEMB-WDL/TUTORIAL/execution
```

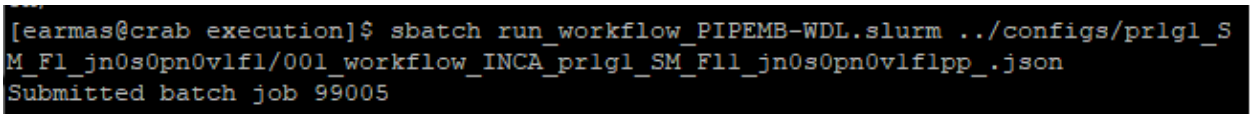
ii. Call Slurm script passing the JSON file as parameter

```
[earmas@crab execution]$ sbatch run_workflow_PIPEMB-WDL.slurm ../configs/prlgl_SM_Fl_jn0s0pn0vlfl/001_workflow_INCA_prlgl_SM_Fll_jn0s0pn0vlflpp_.json
Submitted batch job 99005
```

```
sbatch run_workflow_PIPEMB-WDL.slurm ../configs/pr1g1_SM_F1_jn0s0pn0v1f1/001_workflow_INCA_pr1g1_SM_Fl1_jn0s0pn0v1f1pp_.json
```

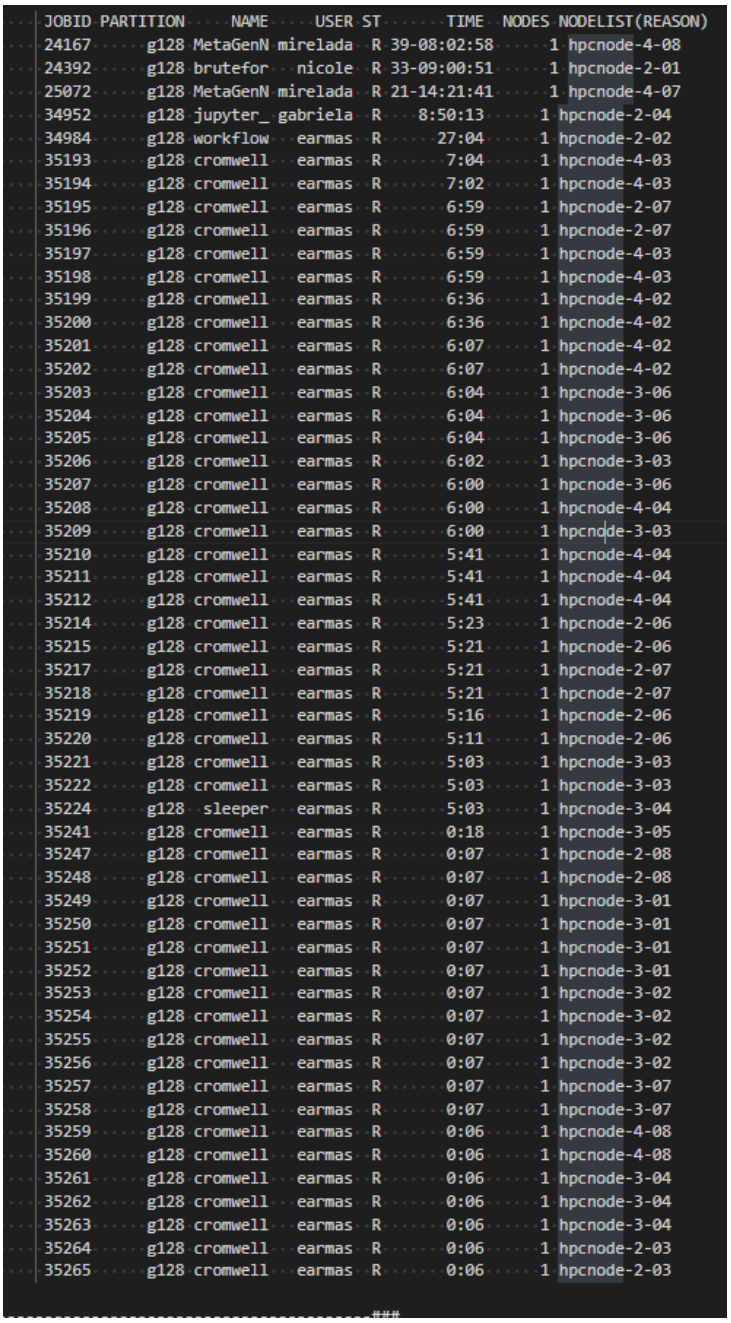
iii. Following the workflow execution:

After starting the execution, the console shows the id number of job corresponding to the current execution. The Cromwell log is save at file named *workflow-<job id number>.log*. This log allows to know the current state of execution and when the workflow finished successfully. An example is illustrated in the following Figure.



In addition, it is possible to see the status of the Slurm queue (squeue command), that is, the currently running jobs and where they are running. Cromwell launches one job per task, and also the main workflow job. An example is shown in the following Figure:

```
[earmas@crab ~]$ cd /data04/tools/PIPEMB/homologacao/PIPEMB-WDL/TUTORIAL/executi
on/
[earmas@crab execution]$ sbatch run_workflow_PIPEMB-WDL.slurm ../configs/prlgl_S
M_Fl_jn0s0pn0vlfl/001_workflow_INCA_prlgl_SM_Fll_jn0s0pn0vlflpp_.json
Submitted batch job 99005
[earmas@crab execution]$ squeue
             JOBID PARTITION     NAME     USER ST       TIME  NODES NODELIST(REA
SON)
             97651      gl28 GATK4_RN carolpou  R 13-22:44:49      1 hpcnode-2-0
2
             98100      gl28     bash izamamed  R 7-23:26:05       1 hpcnode-2-02
             98935      gl28   NETCTL marcopre  R   14:13:59       1 hpcnode-4-07
             99004      gl28     bash gabriela  R    1:14:52       1 hpcnode-4-08
             99005      gl28 workflow   earmas  R       0:02       1 hpcnode-2-01
```



```
JOBID PARTITION     NAME     USER ST       TIME  NODES NODELIST(REASON)
24167      g128 MetaGenN mirelada  R 39-08:02:58      1 hpcnode-4-08
24392      g128 brutefor    nicole  R 33-09:00:51      1 hpcnode-2-01
25072      g128 MetaGenN mirelada  R 21-14:21:41      1 hpcnode-4-07
34952      g128 jupyter_ gabriela  R    8:50:13      1 hpcnode-2-04
34984      g128 workflow    earmas  R      27:04      1 hpcnode-2-02
35193      g128 cromwell    earmas  R       7:04      1 hpcnode-4-03
35194      g128 cromwell    earmas  R       7:02      1 hpcnode-4-03
35195      g128 cromwell    earmas  R       6:59      1 hpcnode-2-07
35196      g128 cromwell    earmas  R       6:59      1 hpcnode-2-07
35197      g128 cromwell    earmas  R       6:59      1 hpcnode-4-03
35198      g128 cromwell    earmas  R       6:59      1 hpcnode-4-03
35199      g128 cromwell    earmas  R       6:36      1 hpcnode-4-02
35200      g128 cromwell    earmas  R       6:36      1 hpcnode-4-02
35201      g128 cromwell    earmas  R       6:07      1 hpcnode-4-02
35202      g128 cromwell    earmas  R       6:07      1 hpcnode-4-02
35203      g128 cromwell    earmas  R       6:04      1 hpcnode-3-06
35204      g128 cromwell    earmas  R       6:04      1 hpcnode-3-06
35205      g128 cromwell    earmas  R       6:04      1 hpcnode-3-06
35206      g128 cromwell    earmas  R       6:02      1 hpcnode-3-03
35207      g128 cromwell    earmas  R       6:00      1 hpcnode-3-06
35208      g128 cromwell    earmas  R       6:00      1 hpcnode-4-04
35209      g128 cromwell    earmas  R       6:00      1 hpcnode-3-03
35210      g128 cromwell    earmas  R       5:41      1 hpcnode-4-04
35211      g128 cromwell    earmas  R       5:41      1 hpcnode-4-04
35212      g128 cromwell    earmas  R       5:41      1 hpcnode-4-04
35214      g128 cromwell    earmas  R       5:23      1 hpcnode-2-06
35215      g128 cromwell    earmas  R       5:21      1 hpcnode-2-06
35217      g128 cromwell    earmas  R       5:21      1 hpcnode-2-07
35218      g128 cromwell    earmas  R       5:21      1 hpcnode-2-07
35219      g128 cromwell    earmas  R       5:16      1 hpcnode-2-06
35220      g128 cromwell    earmas  R       5:11      1 hpcnode-2-06
35221      g128 cromwell    earmas  R       5:03      1 hpcnode-3-03
35222      g128 cromwell    earmas  R       5:03      1 hpcnode-3-03
35224      g128  sleeper    earmas  R       5:03      1 hpcnode-3-04
35241      g128 cromwell    earmas  R       0:18      1 hpcnode-3-05
35247      g128 cromwell    earmas  R       0:07      1 hpcnode-2-08
35248      g128 cromwell    earmas  R       0:07      1 hpcnode-2-08
35249      g128 cromwell    earmas  R       0:07      1 hpcnode-3-01
35250      g128 cromwell    earmas  R       0:07      1 hpcnode-3-01
35251      g128 cromwell    earmas  R       0:07      1 hpcnode-3-01
35252      g128 cromwell    earmas  R       0:07      1 hpcnode-3-01
35253      g128 cromwell    earmas  R       0:07      1 hpcnode-3-02
35254      g128 cromwell    earmas  R       0:07      1 hpcnode-3-02
35255      g128 cromwell    earmas  R       0:07      1 hpcnode-3-02
35256      g128 cromwell    earmas  R       0:07      1 hpcnode-3-02
35257      g128 cromwell    earmas  R       0:07      1 hpcnode-3-07
35258      g128 cromwell    earmas  R       0:07      1 hpcnode-3-07
35259      g128 cromwell    earmas  R       0:06      1 hpcnode-4-08
35260      g128 cromwell    earmas  R       0:06      1 hpcnode-4-08
35261      g128 cromwell    earmas  R       0:06      1 hpcnode-3-04
35262      g128 cromwell    earmas  R       0:06      1 hpcnode-3-04
35263      g128 cromwell    earmas  R       0:06      1 hpcnode-3-04
35264      g128 cromwell    earmas  R       0:06      1 hpcnode-2-03
35265      g128 cromwell    earmas  R       0:06      1 hpcnode-2-03
```

iv. View final outputs:

The configured output directory is /data04/tools/PIPEMB/homologacao/PIPEMB-WDL/TUTORIAL/results/001. The final outputs will appear in this directory, containing the corresponding subdirectories and files for the executed workflow phases.