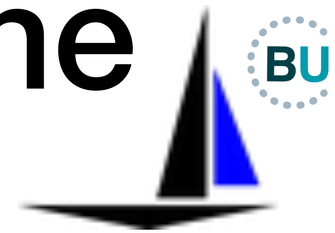
PO: Setup varca pipeline

https://gitlab.com/bu_cnio/varca



CNIO Bioinformatics Unit › varca

varca

Project ID: 12486221

https://gitlab.com/bu_cnio/varca

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141 Commits **2** Branches **0** Tags **809 KB** Files **10.5 MB** Storage

A Snakemake pipeline based on the GATK best-practices workflow

pipeline failed snakemake ≥5.1.5

master varca History Find file Clone

Update README.md
Elena Piñeiro authored 51 minutes ago

254f37fd

README MIT License CI/CD configuration

Name	Last commit	Last update
.test	Add VEP for variant annotation	1 year ago
envs	minimum Snakemake version requirement	1 week ago
img	Add logo to README	1 year ago
report	Add VCF to report.	3 years ago
rules	minimum Snakemake version requirement	1 week ago

Mölder, Felix et al. “Sustainable data analysis with Snakemake.”
F1000Research vol. 10 33. 19 Apr. 2021

Snakemake **is a workflow management system** that allows:

Readability: each **rule** describes a step in an analysis defining how to obtain **output files** from **input files**. Dependencies between rules are determined automatically.

Portability: all software dependencies of each workflow step are **automatically deployed upon execution**.

Modularization: rapidly implement analysis steps via direct script and jupyter notebook integration. Easily create and employ re-usable tool wrappers and **split your data analysis into well-separated modules**.

Transparency: **automatic, interactive, self-contained reports** ensure full transparency from results down to used steps, parameters, code, and software.

Scalability: **workflows scale** from single to multicore, clusters or the cloud.

HOW TO INSTALL varca


```
$ git clone https://gitlab.com/bu_cnio/varca.git
Cloning into 'varca'...
remote: Enumerating objects: 760, done.
remote: Counting objects: 100% (275/275), done.
remote: Compressing objects: 100% (133/133), done.
remote: Total 760 (delta 214), reused 167 (delta 140), pack-reused 485
Receiving objects: 100% (760/760), 1.40 MiB | 12.12 MiB/s, done.
Resolving deltas: 100% (478/478), done.
$
```

HOW TO INSTALL Snakemake

https://snakemake.readthedocs.io/en/stable/getting_started/installation.html

The recommended way is via **Conda/Mamba**

Package, dependency and environment management for any language.
Open source.
Runs on Windows, macOS and Linux.
Installs, runs and updates packages and their dependencies.
Conda easily creates, saves, loads and switches between environments on your local computer.

Mamba is a **faster** reimplementation of the conda package manager

```
$ conda install -n base -c conda-forge mamba
$ conda activate base
(base)$ mamba create -c conda-forge -c bioconda -n snakemake snakemake
```

Running varca: dry run

- Download test data: test.zip
- Uncompress the directory
- Copy test content into varca directory

```
(base) $ cp -r path_to_test/test/* path_to_varca/varca
```

- Activate the snakemake environment

```
(base) $ conda activate snakemake
```

Running varca: dry run and load environments run

- Test dry run: this will tell you if you're good to go!

```
(snakemake) $ snakemake --use-conda —n
Building DAG of jobs...
Job stats:
job                         count    min threads    max threads
-------------------------  -------  -------------  -------------
all                             1              1              1
bed_to_interval                 1              1              1
bwa_idx_genome                  1              1              1
fastqc                          4              1              1
filter_mutect_2                 1              1              1
filter_mutect_calls             1              1              1
genome_dict                     1              1              1
```

- Run varca with --conda-create-envs-only: this will load the environments with the needed dependencies

```
(snakemake) $ snakemake --use-conda --cores 2 --conda-create-envs-only
```

Configuration files

2 configuration files with the **samples definition**

1 configuration file with the **contigs** to analyse

1 configuration file with **paths** to additional files used in the process **and parameters**

Configuration files - samples.tsv

group	sample	control
groups of samples	list of samples	MuTect2 calling mode

- No MuTect2 execution:

group	sample	control
1	A	-

unable MuTect2 calling on the sample

Configuration files - samples.tsv

group	sample	control
groups of samples	list of samples	MuTect2 calling mode

- MuTect2 execution in tumor-only mode:

group	sample	control
1	A	A

- MuTect2 execution in tumor-normal mode:

group	sample	control
1	A	B
1	B	-

Configuration files - units.tsv

```
1  sample  unit  platform  fq1  fq2
2  A       1     ILLUMINA  data/reads/a.chr21.1.fq data/reads/a.chr21.2.fq
3  B       1     ILLUMINA  data/reads/b.chr21.1.fq data/reads/b.chr21.2.fq
4  B       2     ILLUMINA  data/reads/b.chr21.1.fq
```

paired-end and single-end definition

sequential to identify multiple sequences for the same sample

Configuration files - contigs.tsv

```
1  chr1
2  chr2
3  chr3
4  chr4
5  chr5
6  chr6
7  chr7
8  chr8
9  chr9
10 chr10
11 chr11
12 chr12
13 chr13
14 chr14
15 chr15
16 chr16
17 chr17
18 chr18
19 chr19
20 chr20
21 chr21
22 chr22
23 chrX
24 chrY
25 chrM
```

~3300 contigs in hg38

```
@SQ     SN:chr1_KI270708v1_random       LN:127682       M5:1e95e047b98ed92148dd84d6c037158c
@SQ     SN:chr1_KI270709v1_random       LN:66860        M5:4e2db2933ea96aee8dab54af60ecb37d
@SQ     SN:chr1_KI270710v1_random       LN:40176        M5:9949f776680c6214512ee738ac5da289
@SQ     SN:chr1_KI270711v1_random       LN:42210        M5:af383f98cf4492c1f1c4e750c26cbb40
@SQ     SN:chr1_KI270712v1_random       LN:176043       M5:c38a0fecae6a1838a405406f724d6838
@SQ     SN:chr1_KI270713v1_random       LN:40745        M5:cb78d48cc0adbc58822a1c6fe89e3569
@SQ     SN:chr1_KI270714v1_random       LN:41717        M5:42f7a452b8b769d051ad738ee9f00631
@SQ     SN:chr2_KI270715v1_random       LN:161471       M5:b65a8af1d7bbb7f3c77eea85423452bb
@SQ     SN:chr2_KI270716v1_random       LN:153799       M5:2828e63b8edc5e845bf48e75fbad2926
@SQ     SN:chr3_GL000221v1_random       LN:155397       M5:3238fb74ea87ae857f9c7508d315babb
@SQ     SN:chr4_GL000008v2_random       LN:209709       M5:a999388c587908f80406444cebe80ba3
@SQ     SN:chr5_GL000208v1_random       LN:92689        M5:aa81be49bf3fe63a79bdc6a6f279abf6
@SQ     SN:chr9_KI270717v1_random       LN:40062        M5:796773a1ee67c988b4de887addbed9e7
@SQ     SN:chr9_KI270718v1_random       LN:38054        M5:b0c463c8efa8d64442b48e936368dad5
@SQ     SN:chr9_KI270719v1_random       LN:176845       M5:cd5e932cfc4c74d05bb64e2126873a3a
@SQ     SN:chr9_KI270720v1_random       LN:39050        M5:8c2683400a4aeeb40abff96652b9b127
@SQ     SN:chr11_KI270721v1_random      LN:100316       M5:9654b5d3f36845bb9d19a6dbd15d2f22
@SQ     SN:chr14_GL000009v2_random      LN:201709       M5:862f555045546733591ff7ab15bcecbe
@SQ     SN:chr14_GL000225v1_random      LN:211173       M5:63945c3e6962f28ffd469719a747e73c
@SQ     SN:chr14_KI270722v1_random      LN:194050       M5:51f46c9093929e6edc3b4dfd50d803fc
@SQ     SN:chr14_GL000194v1_random      LN:191469       M5:6ac8f815bf8e845bb3031b73f812c012
@SQ     SN:chr14_KI270723v1_random      LN:38115        M5:74a4b480675592095fb0c577c515b5df
```

Configuration files - config.yaml

```yaml
samples: samples.tsv
units: units.tsv
contigs: contigs.tsv

outdir: "out"
logdir: "log"

ref:
  # Genome database of snpeff to be used in the annotation with this resource. Available databases can be checked with java -jar snpEff.jar databases
  name: GRCh38.86
  # Path to the reference genome, ideally as it is provided by the GATK bundle.
  genome: /home/epineiro/REFERENCES/Homo_sapiens/HG38/hg38.fa
  # Path to the directory with the reference indexes for the aligment. Indexes will be retrieve from this directory or created in it if they do not ex
  genome_idx: /home/epineiro/REFERENCES/Homo_sapiens/HG38/indexes/bwa_mem2/
  # Path to any database of known variants, ideally as it is provided by the GATK bundle.
  known-variants: /home/epineiro/REFERENCES/Homo_sapiens/HG38/dbsnp_151.hg38.vcf
```

ref Reference Genome and known variants file

Configuration files - config.yaml

```
18  filtering:
19    # Set to true in order to apply machine learning based recalibration of
20    # quality scores instead of hard filtering.
21    vqsr: false
22    hard:
23      # hard filtering as outlined in GATK docs
24      # (https://gatkforums.broadinstitute.org/gatk/discussion/2806/howto-apply-hard-filters-to-a-call-set)
25      snvs:
26        "QD < 2.0 || QUAL < 100.0 || DP < 50.0 || SOR > 3.0 || FS > 60.0 || MQ < 40.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0"
27      indels:
28        "QD < 2.0 || QUAL < 100 || DP < 50.0 || FS > 200.0 || ReadPosRankSum < -20.0"
29    mutect:
30      #depth of coverage threshold to apply to variants identified with MuTect2
31      depth: "DP < 30"
32      #apply LearnReadOrientationModel to filter out read orientation artifacts
33      lrom: false
34
```

filtering Variant filtering system and parameters for hard filtering

Configuration files - config.yaml

processing

Remove duplicates in the alignment?

```
34
35 processing:
36   remove-duplicates: true
37   # Uncomment and point to a bed file with, e.g., captured regions if necessary,
38   # see https://gatkforums.broadinstitute.org/gatk/discussion/4133/when-should-i-use-l-to-pass-in-a-list-of-intervals.
39   restrict-regions: /home/epineiro/REFERENCES/Homo_sapiens/HG38/S04380219_Padded_v5_hg38_varca.bed
40   # If regions are restricted, uncomment this to enlarge them by the given value in order to include
41   # flanking areas.
42   # region-padding: 100
43
```

Restrict variant calling to specific regions?

Region padding?

Configuration files - config.yaml

params | gatk

Configuration parameters for GATK algorithms

```yaml
params:
  gatk:
    HaplotypeCaller: ""
    BaseRecalibrator: ""
    GenotypeGVCFs: ""
    #If vqsr set to true, fill the following section with the required information (see VariantRecalibrator documentation in GATK)
    VariantRecalibrator:
      #paths to the necessary resources
      hapmap: "/home/epineiro/REFERENCES/Homo_sapiens/HG38/resources_broad_hg38_v0_hapmap_3.3.hg38.vcf.gz"
      omni: "/home/epineiro/REFERENCES/Homo_sapiens/HG38/resources_broad_hg38_v0_1000G_omni2.5.hg38.vcf.gz"
      g1k: "/home/epineiro/REFERENCES/Homo_sapiens/HG38/resources_broad_hg38_v0_1000G_phase1.snps.high_confidence.hg38.vcf.gz"
      dbsnp: "/home/epineiro/REFERENCES/Homo_sapiens/HG38/resources_broad_hg38_v0_Homo_sapiens_assembly38.dbsnp138.vcf"
      #paths to the resource indexes
      aux: ["/home/epineiro/REFERENCES/Homo_sapiens/HG38/resources_broad_hg38_v0_hapmap_3.3.hg38.vcf.gz.tbi",
            "/home/epineiro/REFERENCES/Homo_sapiens/HG38/resources_broad_hg38_v0_1000G_omni2.5.hg38.vcf.gz.tbi",
            "/home/epineiro/REFERENCES/Homo_sapiens/HG38/resources_broad_hg38_v0_1000G_phase1.snps.high_confidence.hg38.vcf.gz.tbi",
            "/home/epineiro/REFERENCES/Homo_sapiens/HG38/resources_broad_hg38_v0_Homo_sapiens_assembly38.dbsnp138.vcf.idx"]
      #set definition for each resource
      parameters: {"hapmap": {"known": False, "training": True, "truth": True, "prior": 15.0},
                   "omni":   {"known": False, "training": True, "truth": False, "prior": 12.0},
                   "g1k":    {"known": False, "training": True, "truth": False, "prior": 10.0},
                   "dbsnp":  {"known": True, "training": False, "truth": False, "prior": 2.0}}
      #Names of the annotations used for calculations
      annotation: ["QD", "MQ", "MQRankSum", "ReadPosRankSum", "FS", "SOR"]
      extra: ""
```

Configuration files - config.yaml

params

picard

Configuration parameter for picard

```
    extra:
  picard:
    MarkDuplicates: "REMOVE_DUPLICATES=true"
```

Configuration files - config.yaml

params trimmomatic Configuration parameters for trimmomatic

```
71  trimmomatic:
72    pe:
73      trimmer:
74        # See trimmomatic manual for adding additional options, e.g. for adapter trimming.
75        - "LEADING:3"
76        - "TRAILING:3"
77        - "SLIDINGWINDOW:4:15"
78        - "MINLEN:36"
79    se:
80      trimmer:
81        # See trimmomatic manual for adding additional options, e.g. for adapter trimming.
82        - "LEADING:3"
83        - "TRAILING:3"
84        - "SLIDINGWINDOW:4:15"
85        - "MINLEN:36"
86
```

Configuration files - config.yaml

annotation

Parameters for VEP annotation

```
86
87 annotation:
88   vep:
89     # If set to true, a cache directory must be created using the instructions of VEP. Using a cache allows for a fastest and most efficient way to u
90     cache: true
91     #Indicate the version of the cache if cache is set to true. e.g. 100. This version must match the one indicated in the vep environment (vep.yaml)
92     cache_version: 100
93     #Indicate the path to the cache files if cache is set to true
94     cache_directory: /home/epineiro/Programs/VEP/VEP100/.vep/
95     #Indicate the assembly version of the reference genome
96     assembly: GRCh38
97     #Indicate the annotations to include with the VEP execution and additional parameters. See the documentation (https://www.ensembl.org/info/docs/t
98     annotations: "--sift b --polyphen b --ccds --uniprot --hgvs --symbol --numbers --domains --regulatory --canonical --protein --biotype --uniprot --
99
```

Configuration files - config.yaml

resources

Machine requirements

```
resources:
    default:
        threads: 1
        mem: 32000
        walltime: 720
    snpeff:
        mem: 8000
        walltime: 480
    call_variants:
        mem: 10000
        threads: 4
        walltime: 480
    combine_calls:
        mem: 8000
        threads: 1
        walltime: 480
    recalibrate_calls:
        mem: 8000
        walltime: 480
    hard_filter_calls:
        mem: 8000
        walltime: 480
    trim_reads:
        mem: 4000
        threads: 16
        walltime: 480
    map_reads:
        mem: 32000
        threads: 8
        walltime: 480
```

Extra: In case you need to install conda

- Go to https://docs.conda.io/en/latest/miniconda.html#linux-installers
- Download the miniconda bundle (choose the optimal version for your computer)

```
$ wget https://repo.anaconda.com/miniconda/Miniconda3-py39_4.10.3-Linux-x86_64.sh
```

- In the directory where the file Miniconda3-py39_4.10.3-Linux-x86_64.sh

```
$ bash Miniconda3-py39_4.10.3-Linux-x86_64.sh
```

- Once the installation is over, restart the terminal.