Institut Pasteur

Sequana: a set of flexible genomic pipelines for processing and reporting NGS analysis

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Motivation

NGS at Biomics (Sean Kennedy)

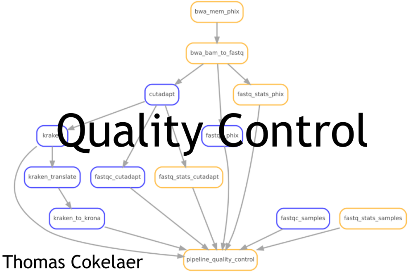
Development driven by the Biomics Pole at Pasteur Institute, which involves many aspects of NGS including :

https://research.pasteur.fr/en/team/biomics/

- De novo and targeted sequencing of viruses, prokaryotes and eukaryotes
- Variant (SNP, indel, large rearrangements) detection
- Human and Mouse SNP detection by array
- Transcriptional analysis (RNA-Seq) for both prokaryotes and eukaryotes
- 16S and deep-sequencing metagenomic studies (mouse, human, and other environments)
- Bottom-up whole proteomic analysis and quantification
- Analysis of a wide range of post-translational modifications
- Determination of the dynamics of protein complexes.
- Epigenetics (CHIP-Seq, methylation studies)
- Projects involving two or more techniques (i.e. proteogenomics, single-cell DNA/RNA analysis)

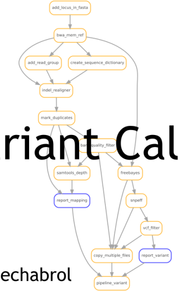
Pipelines

Pipelines available in Sequana



Quality Control

Thomas Cokelaer



Variant Calling

Dimitri Desvillechabrol

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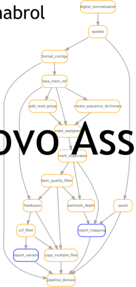
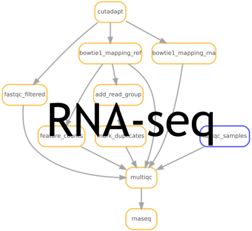


De Novo Assembly

Rachel Legendre



RNA-seq

Rules specificity: input and output are variables

- Rules are generic and easily reusable

mark_duplicates.rules

```
rule mark_duplicates:
    input:
        __mark_duplicates__input
    output:
        bam = __mark_duplicates__output,
        metrics = __mark_duplicates__metrics
    log:
        out = __mark_duplicates__log_std,
        err = __mark_duplicates__log_err
```

Dynamics rules

- Each rule must be unique in a pipeline

Some pipelines must use multiple times one rules like fastqc in quality control pipeline

- These rules are templatized to become dynamic:

quality_control.rules

```
exec(open(sequana.modules["fastqc"], "r").read())
        ...
include: fastqc_dynamic("samples", manager)
        ...
include: fastqc_dynamic("phix", manager)
        ...
include: fastqc_dynamic(adapter_removal, manager)
```

Usage

Using command line

- One command line to initiate the pipeline

Shell

```
sequana --pipeline variant_calling \
        --input-directory path/to/sample/ \
        --reference sequence.fasta \
        --output-directory analysis/
```

- The sequana executable creates a directory with the project name
- The directory contains all the necessary files (config, snakefile)

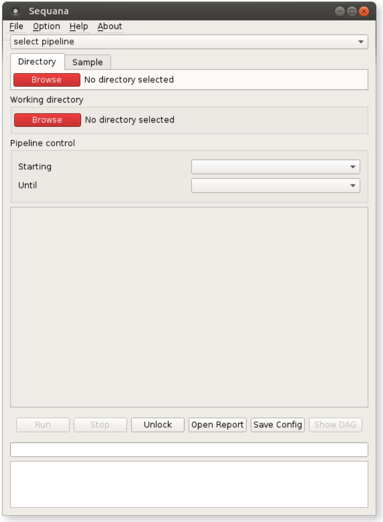
Continuous Integration

Versioning, Test and Documentation

- Sequana is available on GitHub (github.com/sequana/sequana)
- Continuous Integration on Travis with 60 tests with 60% coverage
- Documentation available on sequana.readthedocs.org .
  - Uses Sphinx (RST syntax) to document the source code and provides user guide.
  - Updated automatically at each commits

GUI

GUI to simplify the usage of snakemake

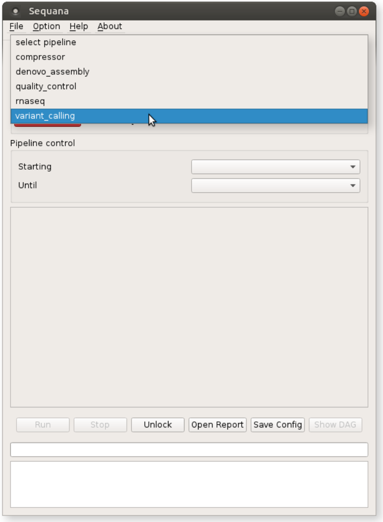


Directory: No directory selected

Working directory: No directory selected

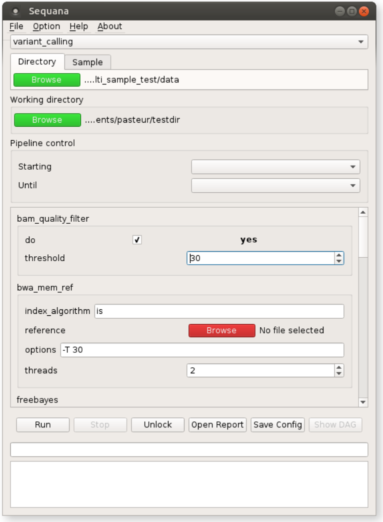
- Interface developed with PyQT5 and python
- Wrap our snakemake pipelines to ease the usage
- Usable on our cluster, which allows X11

GUI to simplify the usage of snakemake



1. Choose a pipeline

GUI to simplify the usage of snakemake



variant_calling

....lti_sample_test/data

Working directory

....ents/pasteur/testdir

Pipeline control

Starting

Until

bam_quality_filter

do ☑ yes

threshold 30

bwa_mem_ref

index_algorithm is

reference No file selected

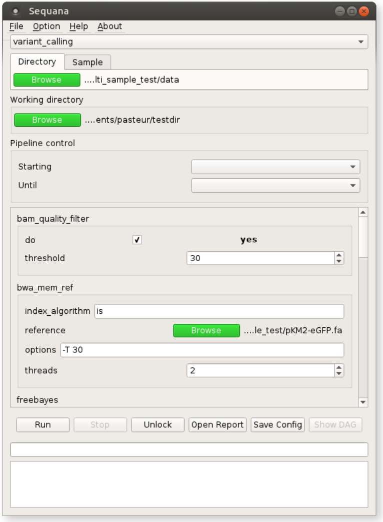
options -T 30

threads 2

freebayes

1. Choose a pipeline
2. Set input and output

GUI to simplify the usage of snakemake



variant_calling

Directory | Sample

....lti_sample_test/data

Working directory

....ents/pasteur/testdir

Pipeline control

Starting

Until

bam_quality_filter

do ☑ yes

threshold 30

bwa_mem_ref

index_algorithm is

reference ....le_test/pKM2-eGFP.fa

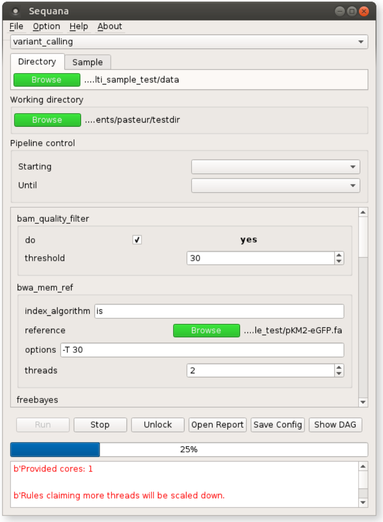
options -T 30

threads 2

freebayes

1. Choose a pipeline
2. Set input and output
3. Fill the config formular

GUI to simplify the usage of snakemake



variant_calling

....lti_sample_test/data

Working directory

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Pipeline control

Starting

Until

bam_quality_filter

do ☑ yes

threshold 30

bwa_mem_ref

index_algorithm is

reference ....le_test/pKM2-eGFP.fa

options -T 30

threads 2

freebayes

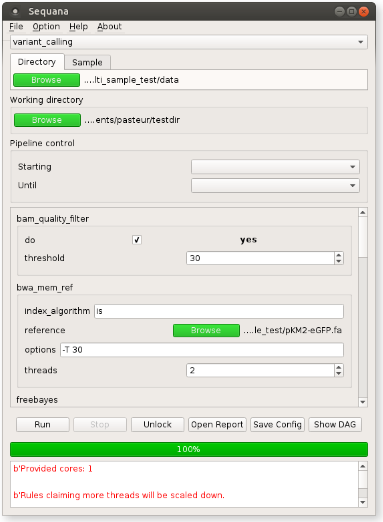
25%

```
b'Provided cores: 1

b'Rules claiming more threads will be scaled down.
```

1. Choose a pipeline
2. Set input and output
3. Fill the config formular
4. Run the pipeline

GUI to simplify the usage of snakemake



variant_calling

Browse ....lti_sample_test/data

Working directory: Browse ....ents/pasteur/testdir

Starting

Until

bam_quality_filter

do ☑ yes

threshold 30

bwa_mem_ref

index_algorithm is

reference Browse ....le_test/pKM2-eGFP.fa

options -T 30

threads 2

freebayes

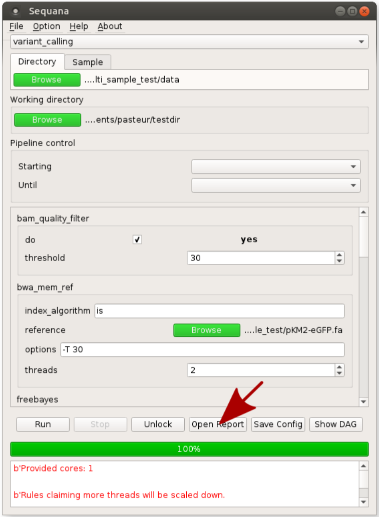
100%

```
b'Provided cores: 1

b'Rules claiming more threads will be scaled down.
```

1. Choose a pipeline
2. Set input and output
3. Fill the config formular
4. Run the pipeline
5. Finished !

GUI to simplify the usage of snakemake



variant_calling

....lti_sample_test/data

Working directory

....ents/pasteur/testdir

Pipeline control

Starting

Until

bam_quality_filter

do ☑ yes

threshold 30

bwa_mem_ref

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reference ....le_test/pKM2-eGFP.fa

options -T 30

threads 2

freebayes

```
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```
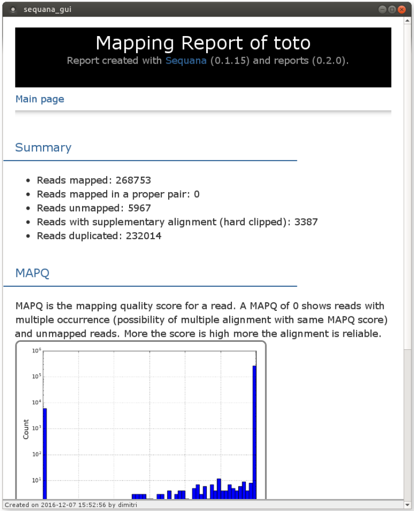



Mapping Report of toto

Report created with Sequana (0.1.15) and reports (0.2.0).

Summary

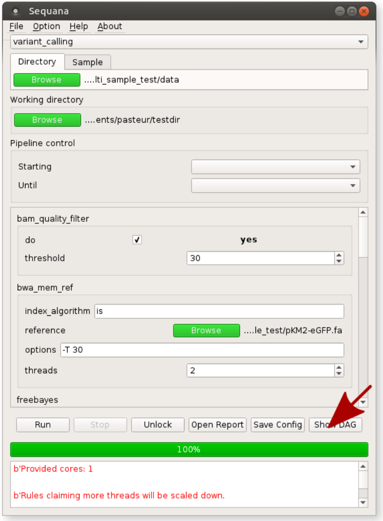
- Reads mapped: 268753
- Reads mapped in a proper pair: 0
- Reads unmapped: 5967
- Reads with supplementary alignment (hard clipped): 3387
- Reads duplicated: 232014

MAPQ

MAPQ is the mapping quality score for a read. A MAPQ of 0 shows reads with multiple occurrence (possibility of multiple alignment with same MAPQ score) and unmapped reads. More the score is high more the alignment is reliable.

Created on 2016-12-07 15:52:56 by dimitri

Ease the pipeline manipulation and viewing



variant_calling

Directory: ....lti_sample_test/data

Working directory: ....ents/pasteur/testdir

Pipeline control

Starting

Until

bam_quality_filter

do ☑ yes

threshold 30

bwa_mem_ref

index_algorithm is

reference ....le_test/pKM2-eGFP.fa

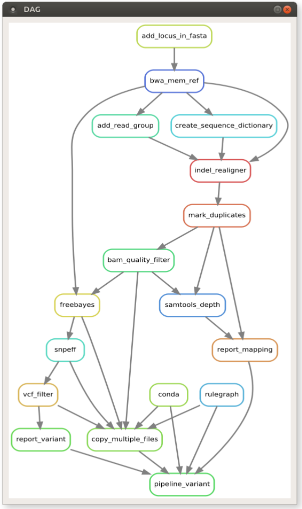
options -T 30

threads 2

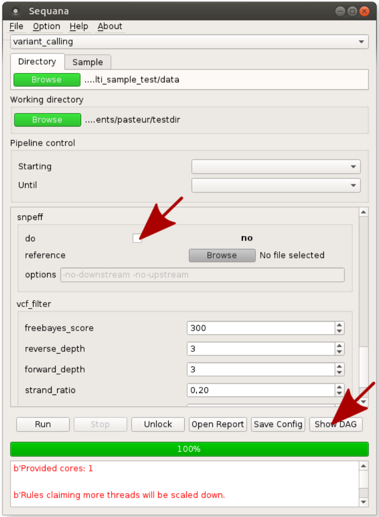
freebayes

100%

```
b'Provided cores: 1

b'Rules claiming more threads will be scaled down.
```



Ease the pipeline manipulation and viewing



Sequana

variant_calling

Directory: ....lti_sample_test/data

Working directory: ....ents/pasteur/testdir

Pipeline control

Starting

Until

snpeff

do □ no

reference: No file selected

options: -no-downstream -no-upstream

vcf_filter

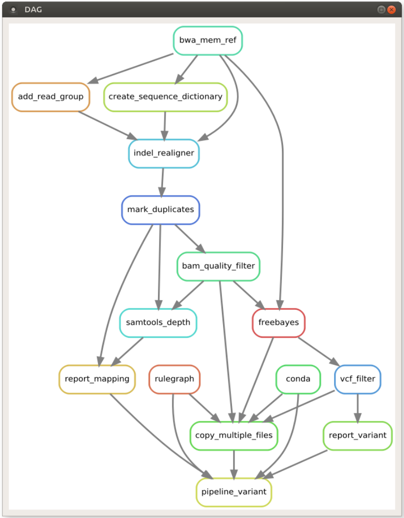
freebayes_score: 300

reverse_depth: 3

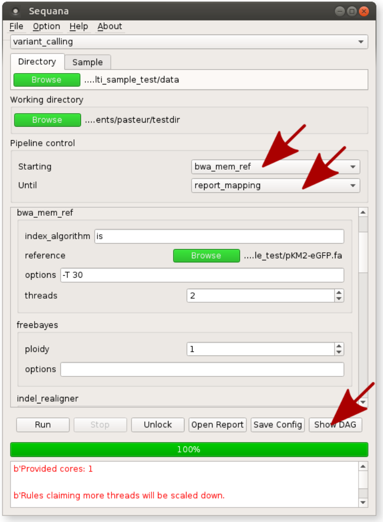
forward_depth: 3

strand_ratio: 0,20

```
b'Provided cores: 1

b'Rules claiming more threads will be scaled down.
```



Ease the pipeline manipulation and viewing



variant_calling

Directory: ....lti_sample_test/data

Working directory: ....ents/pasteur/testdir

Pipeline control

Starting: bwa_mem_ref

Until: report_mapping

bwa_mem_ref

index_algorithm: is

reference: ....le_test/pKM2-eGFP.fa

options: -T 30

threads: 2

freebayes

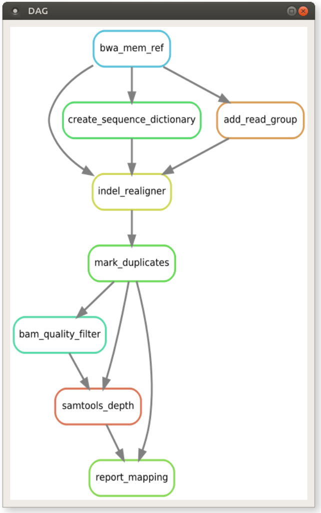
ploidy: 1

options:

indel_realigner

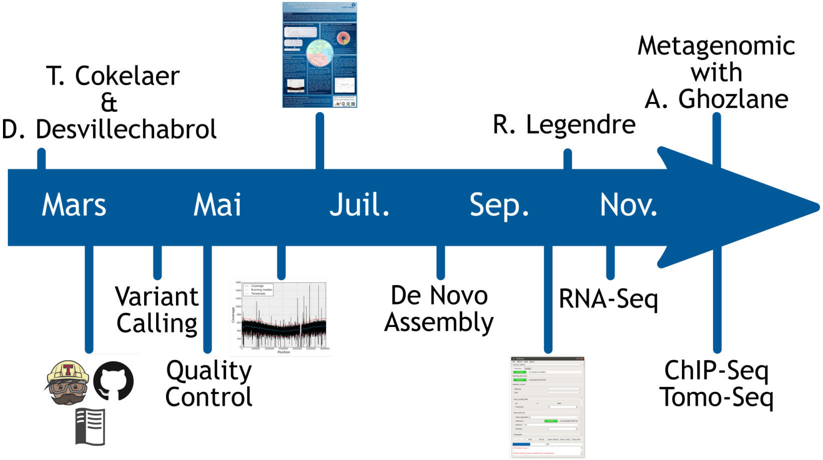
100%

```
b'Provided cores: 1

b'Rules claiming more threads will be scaled down.
```



Summary and Future Directions

Sequana history



[1] Detection and characterization of low and high genome coverage regions using an efficient running median and a double threshold approach. Dimitri Desvillechabrol, Christiane Bouchier, Sean Kennedy, Thomas Cokelaer bioRxiv 092478; doi: http://dx.doi.org/10.1101/092478

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Citech

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