



Genotoul
Bioinfo

Nextflow nf-core/SAREK

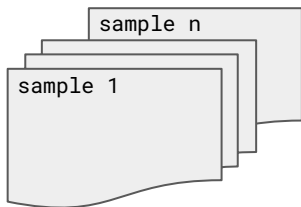


Novembre 2023



- ❖ Context
- ❖ Nextflow / workflow repository (nf-core)
- ❖ Run a workflow
- ❖ Outputs





QC

- `$ fastqc sample 1`
- `$ fastqc sample 2`
- `$ fastqc sample 3`
- ...
- `$ fastqc sample n`

SRUN



SLURM



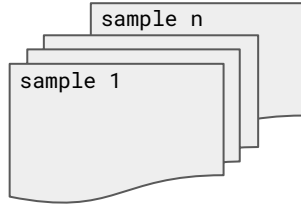
HPC

Workflow example

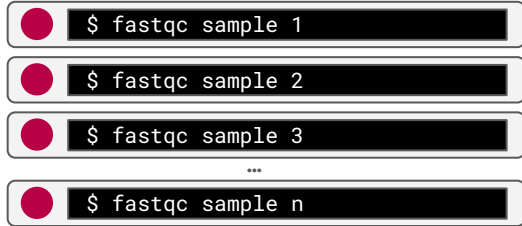




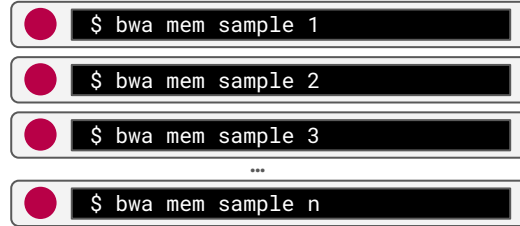
Workflow example



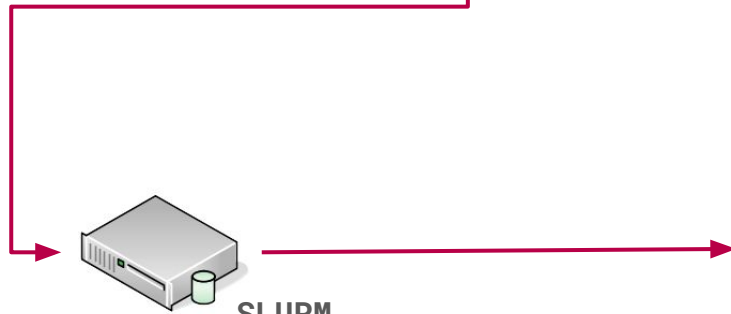
QC



ALN



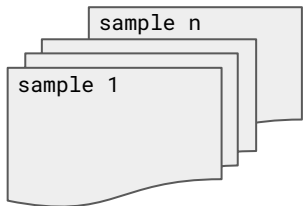
SRUN



HPC



Workflow example



QC

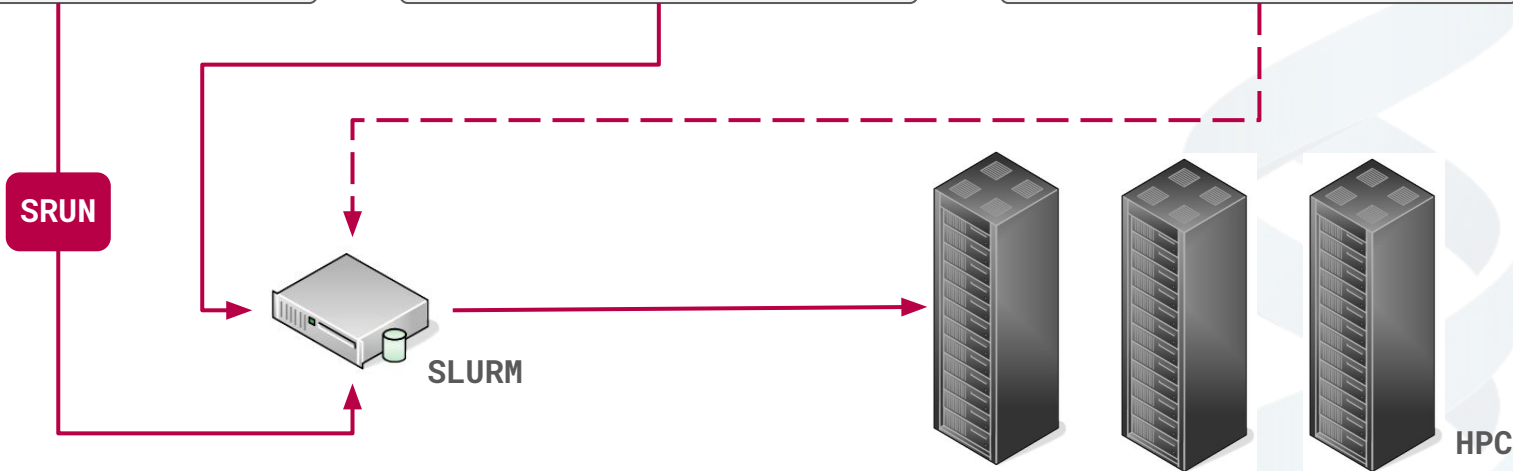
- `$ fastqc sample 1`
- `$ fastqc sample 2`
- `$ fastqc sample 3`
- ...
- `$ fastqc sample n`

ALN

- `$ bwa mem sample 1`
- `$ bwa mem sample 2`
- `$ bwa mem sample 3`
- ...
- `$ bwa mem sample n`

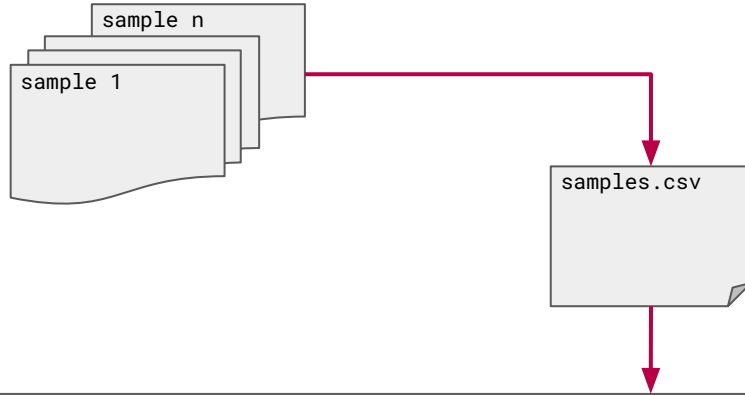
GATK

- `$ gatk MarkDuplicates sample 1`
- `$ gatk MarkDuplicates sample 2`
- `$ gatk MarkDuplicates sample 3`
- ...
- `$ gatk MarkDuplicates sample n`



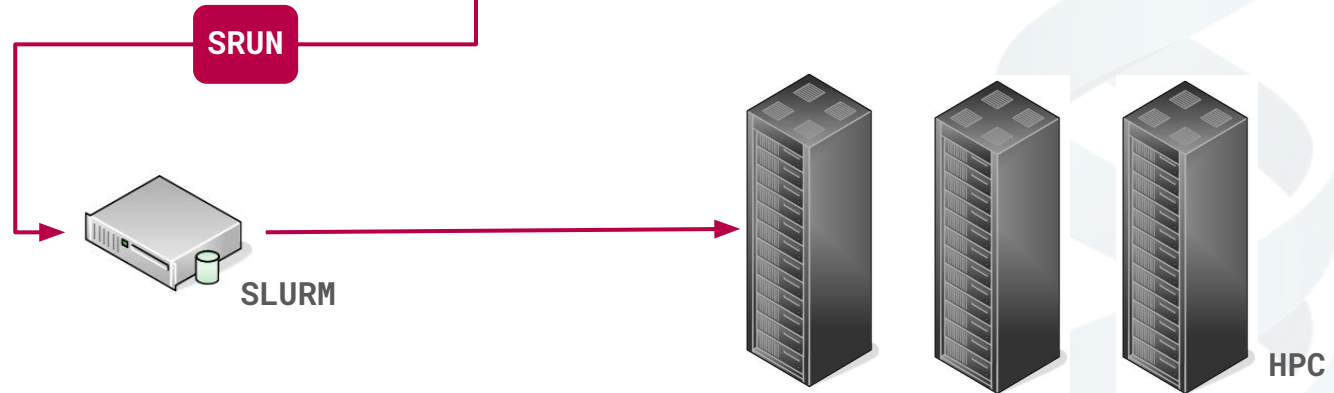


Workflow example



Nextflow

```
$ nextflow run nf-core/sarek --input samples.csv --genome genome.fa --tools haplotypcaller
```





- ❖ Execution and parallelization
- ❖ Reproducibility
- ❖ Workflows **versioning** in a repository
- ❖ **Containers** with dependencies (software)
- ❖ Few manual configuration
- ❖ Same usage on Gentoul, IFB, Amazon...

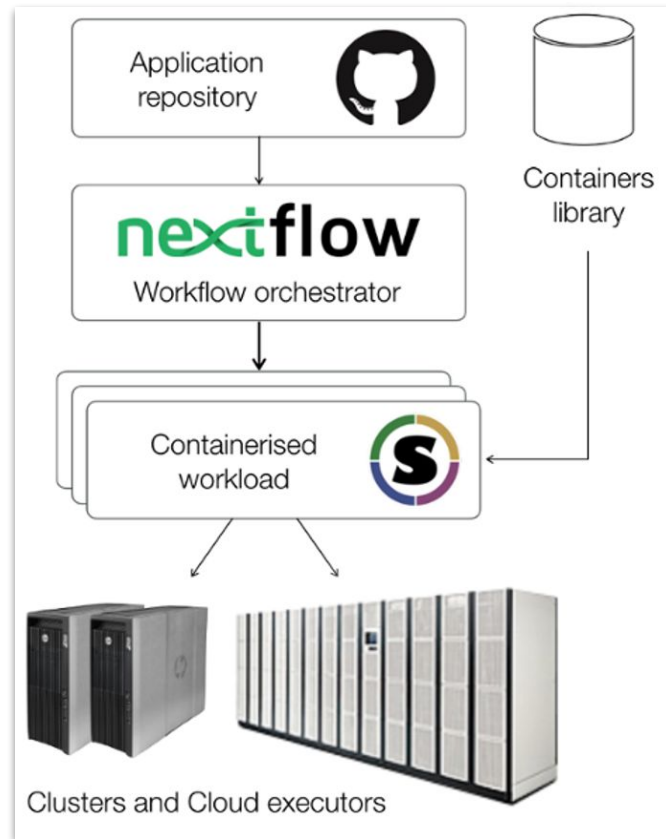




General concepts: nextflow



- ❖ Developed at CRG
- ❖ Java
- ❖ Large user community





General concepts: nextflow



<https://www.nextflow.io/docs/latest>

Nextflow can run a workflow from:

- ❖ a file (.nf)
- ❖ a repository:
 - Github
 - Gitlab
 - BitBucket



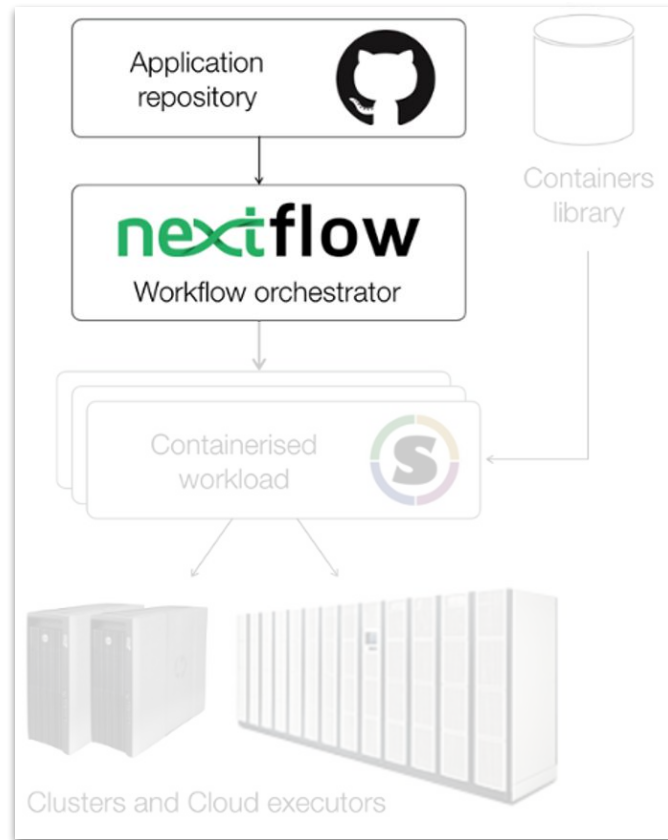


General concepts: repository



<https://nf-co.re/>

nf-core













- ❖ Start of 2018
NGI Stockholm
- ❖ A community effort to collect a curated set of analysis pipelines built using Nextflow

What is nf-core?





Deploy

-  Stable pipelines
-  Centralised configs
-  List and update pipelines
-  Download for offline use

Participate

-  Documentation
-  Slack workspace
-  Twitter updates
-  Hackathons

Develop

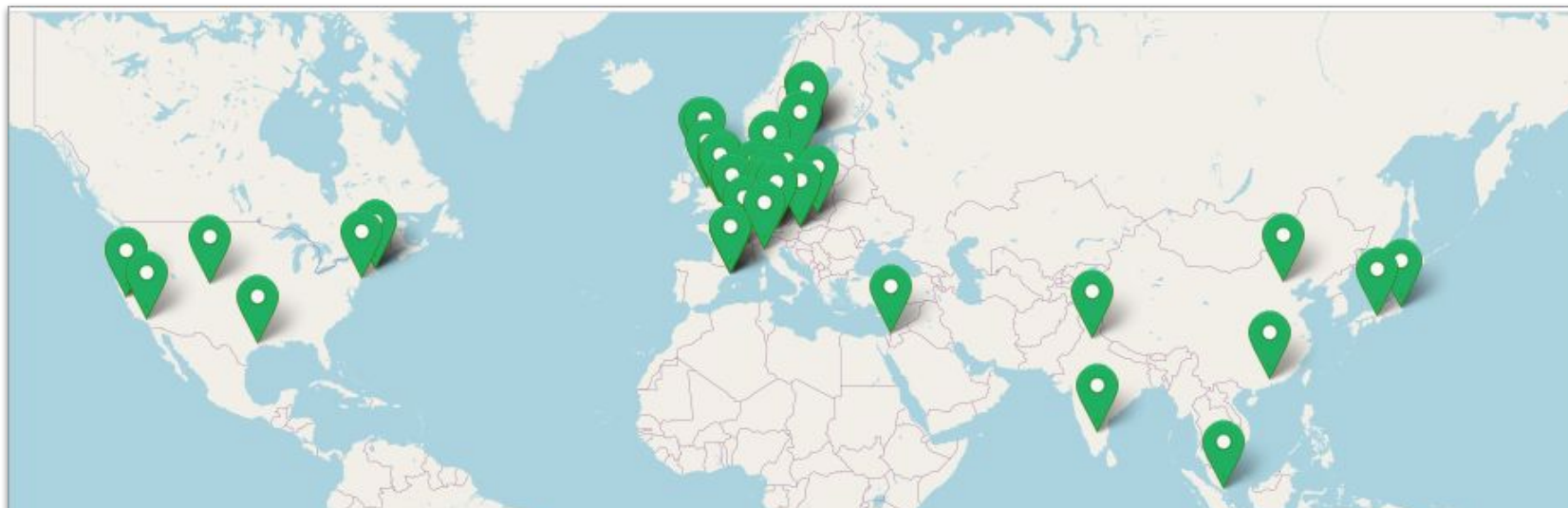
-  Starter template
-  Code guidelines
-  CI code linting and tests
-  Helper tools


Pipelines

Browse the 93 pipelines that are currently available



nf-core



 slack

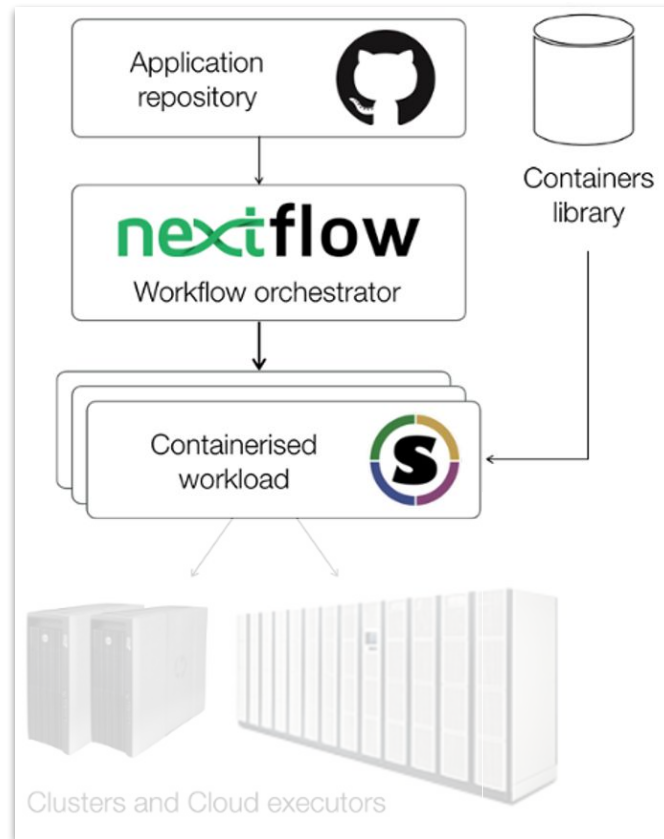
Sign in to nf-core

nfcore.slack.com

You plan to develop workflows?
You can join INRAE mattermost
<https://team.forgemia.inra.fr/tlse-nextflow>



General concepts: containers





General concepts: containers



OR



« allows you to run one or more linux applications inside an isolated and reproducible environment called a container, which shares the linux kernel of the machine you are on »

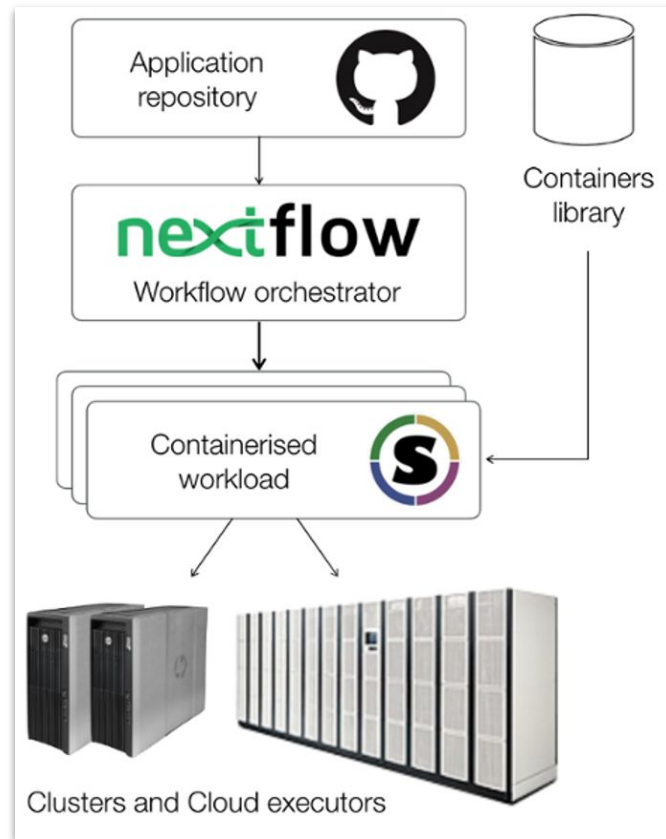
OR



Conda is a package manager and environment management system (based on the system)



General concepts: infrastructure





General concepts: infrastructure



- ❖ On genobioinfo, do only one time (create ~/.nextflow & ~/.singularity link),

```
sh /usr/local/bioinfo/src/NextflowWorkflows/create_nfx_dirs.sh
```

- ❖ load module

```
module load bioinfo/Nextflow/23.04.3
```

- ❖ and use profile

```
nextflow run myWorkflow -profile genotoul
```




❖ Execute a workflow (not a nf-core workflow)

```
$ nextflow run hello
N E X T F L O W ~ version 23.04.3
Pulling nextflow-io/hello ...
  downloaded from https://github.com/nextflow-io/hello.git
Launching `https://github.com/nextflow-io/hello` [stoic_einstein] DSL2 - revision: 1d71f857bb [master]
executor > local (4)
[32/924d04] process > sayHello (1) [100%] 4 of 4 ✓
Ciao world!
Hola world!
Hello world!
Bonjour world!
```



How to run a nf-core workflow?



- ❖ On genobioinfo,

```
module load bioinfo/NextflowWorkflows/nfcore-Nextflow-v23.04.3
```

- ❖ load nextflow, singularity and some env. variable

```
$ module show bioinfo/NextflowWorkflows/nfcore-Nextflow-v23.04.3
-----
/tools/modulefiles/bioinfo/NextflowWorkflows/nfcore-Nextflow-v23.04.3:

module-whatis  {loads the bioinfo/NextflowWorkflows/nfcore-Nextflow-v23.04.3 environment}
module        load devel/java/17.0.6
module        load containers/singularity/3.9.9
module        load bioinfo/Nextflow/23.04.3
module        load devel/python/Python-3.11.1
setenv        NXF_SINGULARITY_CACHEDIR /usr/local/bioinfo/src/NextflowWorkflows/singularity-img/
```



How to run a nf-core workflow?



❖ Call nextflow help

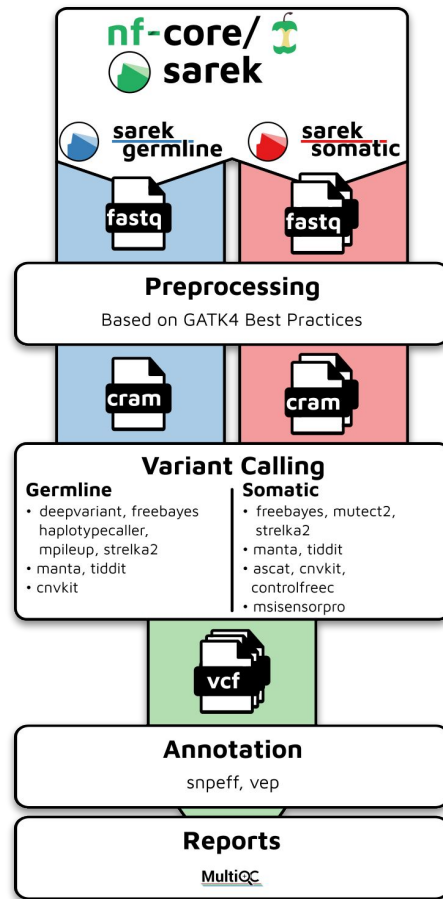
```
nextflow help
```

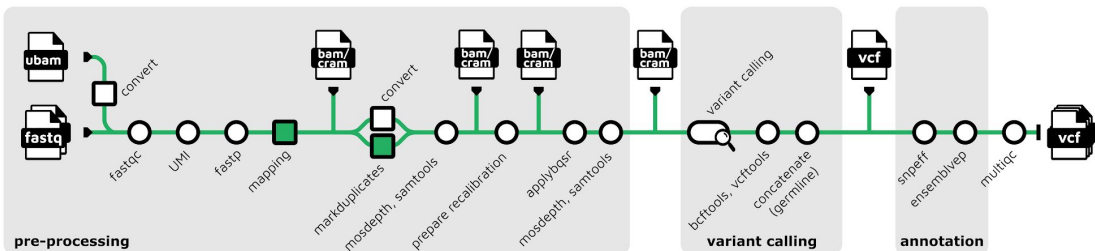
❖ Call nf-core nextflow help

```
$ nextflow run nf-core/sarek -r 3.2.2 --help
N E X T F L O W ~ version 23.04.3
Launching `https://github.com/nf-core/sarek` [insane_lattes] DSL2 - revision: 6ec8c1c945 [3.2.2]
...
  nf-core/sarek v3.2.2-g6ec8c1c
-----
Typical pipeline command:
  nextflow run nf-core/sarek --input samplesheet.csv --genome GATK.GRCh38 -profile docker
...
```

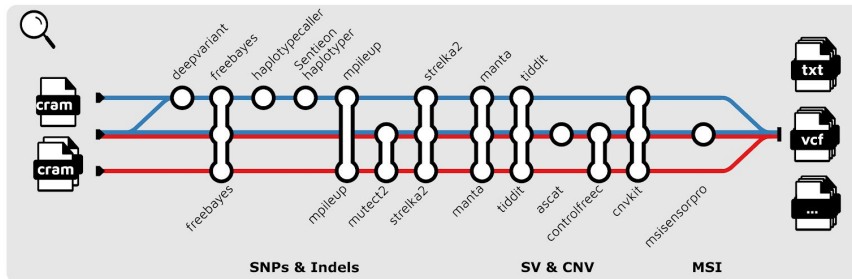


- ❖ Analysis pipeline to detect germline or somatic variants (pre-processing, variant calling and annotation) from WGS / targeted sequencing
- ❖ <https://nf-co.re/sarek>



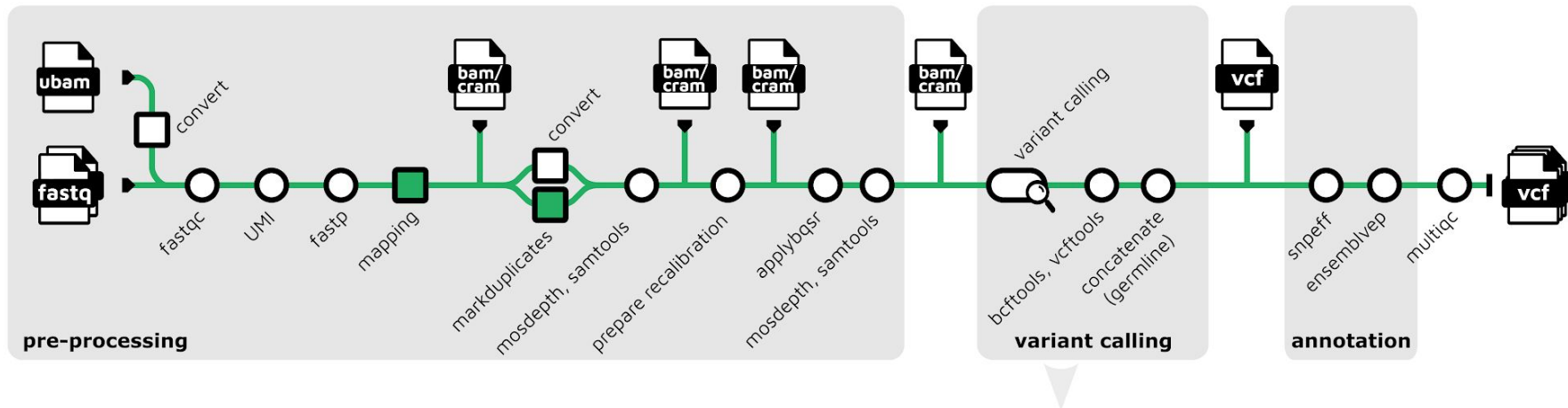


Example analysis pathways



- Mandatory
- Optional
- Optionally Sentieon accelerated
- Core workflow
- Germline variant calling
- Tumor only variant calling
- Tumor-normal pair variant calling

Adapted from: Fellows Yates, James A., et al. PeerJ 9 (2021).



□ Mandatory

○ Optional

■ Optionally Sentieon accelerated

— Core workflow

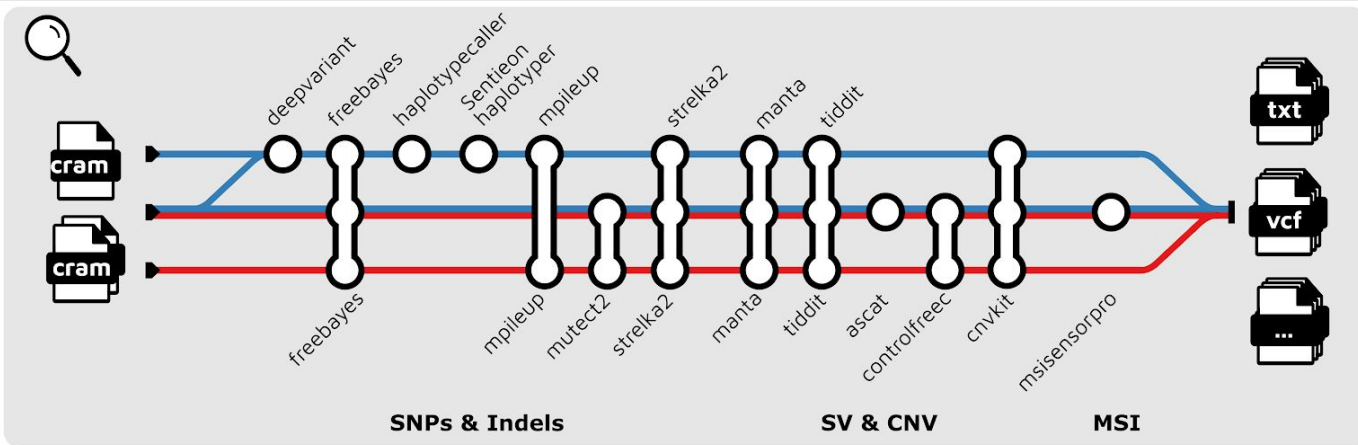
— Germline variant calling

— Tumor only variant calling

— Tumor-normal pair variant calling



Example analysis pathways



□ Mandatory

○ Optional

■ Optionally Sentieon accelerated

— Core workflow

— Germline variant calling

— Tumor only variant calling

— Tumor-normal pair variant calling

Adapted from: Fellows Yates, James A., et al. PeerJ 9 (2021).



- ❖ Sample sheet with fastq pairs:
 - csv format
 - at least 3 columns
 - header line
 - specified with the `--input` parameter
- ❖ Minimal config file:

```
patient, sample, lane, fastq_1, fastq_2  
patient1, test_sample, lane_1, test_R1.fastq.gz, test_R2.fastq.gz
```

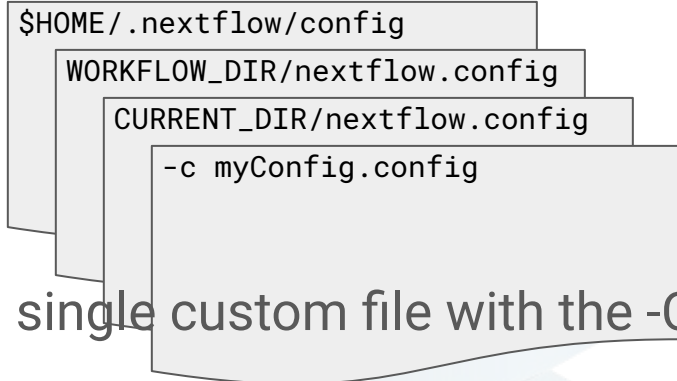



- ❖ You need only a fasta file
- ❖ You can provide known variants
 - either use `--genome` and `--fasta` parameters
 - or configure a `nextflow.config` file

```
params {
  genomes {
    'Gallus_gallus-5.0_25-26' {
      fasta      = "${params.genomes_base}/Gallus_gallus.Gallus_gallus-5.0.dna.toplevel_chr25-26.fa"
      species    = 'Gallus_gallus'
      known_indels = "${params.genomes_base}/Gallus_gallus_incl_consequences_chr25-26.vcf.gz"
    }
  }
}
```



- ❖ The workflow configuration is a merge of several config files found in:
 - user home directory
 - workflow directory
 - current directory



- ❖ To ignore any default configuration, use a single custom file with the `-C` command line option

```
nextflow run nf-core/sarek -r 3.2.2 -C myConfig.config
```

- ❖ To know the used configuration

```
nextflow config nf-core/sarek
```



nf-core/sarek: execution



Use singularity image

input and outdir

jobs launched with slurm

task working directory

complete job

failed job

parallelized tasks

```
N E X T F L O W ~ version 23.04.3
Launching `https://github.com/nf-core/sarek` [ecstatic_lavoisier] DSL2 - revision: 6c0d335e17
[3.2.1]
Core Nextflow options
  revision           : 3.3.2
  runName            : ecstatic_lavoisier
  containerEngine    : singularity
  launchDir          : /work/user/ccabau/nextflow-sarek
  workDir            : /work/user/ccabau/nextflow-sarek/work
  projectDir         : /home/ccabau/.nextflow/assets/nf-core/sarek
  userName           : ccabau
  profile            : genotoul
  configFiles        : /home/ccabau/.nextflow/assets/nf-core/sarek/nextflow.config

Input/output options
  input              : sample.csv
  outdir             : results_chicken

executor > slurm (8)
[0e/b9ecc6] process > get_software_versions           [ 0%] 0 of 1
[a9/d87864] process > BuildBWAindexes (Gallus_gal... [ 0%] 0 of 1
[34/5854df] process > BuildDict (Gallus_gallus.Ga... [ 0%] 0 of 1
[a1/a2e06b] process > BuildFastaFai (Gallus_gallu... [100%] 1 of 1 ✓
[-          ] process > BuildGermlineResourceIndex   -
[6c/b9bb9c] process > BuildKnownIndelsIndex (Gall... [100%] 1 of 1, failed: 1 ✗
[-          ] process > BuildPonIndex                   -
[ca/d7f80b] process > BuildIntervals (Gallus_gall... [ 0%] 0 of 1
[a8/7c8106] process > BaseRecalibrator (chicken-S... [ 50%] 2 of 4
[-          ] process > TrimGalore                       [ 0%] 0 of 1
```



- ❖ All nf-core pipelines have:
 - several directories per step
 - a MultiQC output directory
 - a pipeline_info output directory
 - ...





nf-core/sarek: outputs



```
results_chicken
├── multiqc                                # main HTML reports
│   ├── multiqc_data
│   ├── multiqc_plots
│   └── multiqc_report.html
├── pipeline_info
│   ├── execution_report_2023-11-06_17-56-20.html    # HTML CPU, Memory, Time report
│   ├── execution_timeline_2023-11-06_17-56-20.html  # HTML timeline
│   ├── execution_trace_2023-11-06_17-56-20.txt     # txt trace
│   ├── params_2023-11-06_18-18-20.json
│   ├── pipeline_dag_2023-11-06_17-56-20.html       # workflow graphic representation
│   └── software_versions.yml                       # version of all softwares
├── preprocessing                          # BAM, BAI
│   ├── markduplicates
│   ├── recalibrated
│   └── recal_table
├── reference                              # all indexes to reuse in other pipeline/execution
├── reports                                # TXT or HTML reports for each step
│   ├── bcftools
│   ├── fastqc
│   ├── markduplicates
│   ├── samtools
│   └── vcftools
└── variant_calling                       # calling results per caller and per sample
    ├── deepvariant
    ├── freebayes
    └── haplotypcaller
```

A modular tool to aggregate results from bioinformatics analyses across many samples into a single report.

This report has been generated by the [nf-core/sarek](#) analysis pipeline. For information about how to interpret these results, please see the [documentation](#).

Report generated on 2023-11-06, 18:17 CET based on data in: `/work/user/ccabau/nextflow-sarek-deep/work/d8/37cc491260da8110fec506d45feedf`

Welcome! Not sure where to start? [Watch a tutorial video](#) (6:06) [don't show again](#) ✕

General Statistics

[Copy table](#) [Configure Columns](#) [Plot](#) Showing 19_{r19} rows and 20_{c31} columns.

Sample Name	% Dups	% GC	M Seqs	% Duplication	M Reads After Filtering	GC content	% PF	% Dups	Error rate	M Non-Primary
SRR7062654-1				0.9%	1.9	50.6%	94.9%			
SRR7062654-1.md								2.7%		
SRR7062654-1_1	5.1%	50%	1.0							
SRR7062654-1_2	4.7%	50%	1.0							
SRR7062654.deepvariant										
SRR7062654.md										
SRR7062654.md.cram								0.91%		0.0
SRR7062654.recal										
SRR7062654.recal.cram								0.91%		0.0
SRR7062655-1				1.6%	1.8	50.5%	94.7%			
SRR7062655-1.md								4.3%		
SRR7062655-1_1	6.7%	50%	1.0							
SRR7062655-1_2	6.1%	50%	1.0							



pipeline_info/execution_report



Nextflow Report Summary Resources Tasks

[disturbed_perlman]

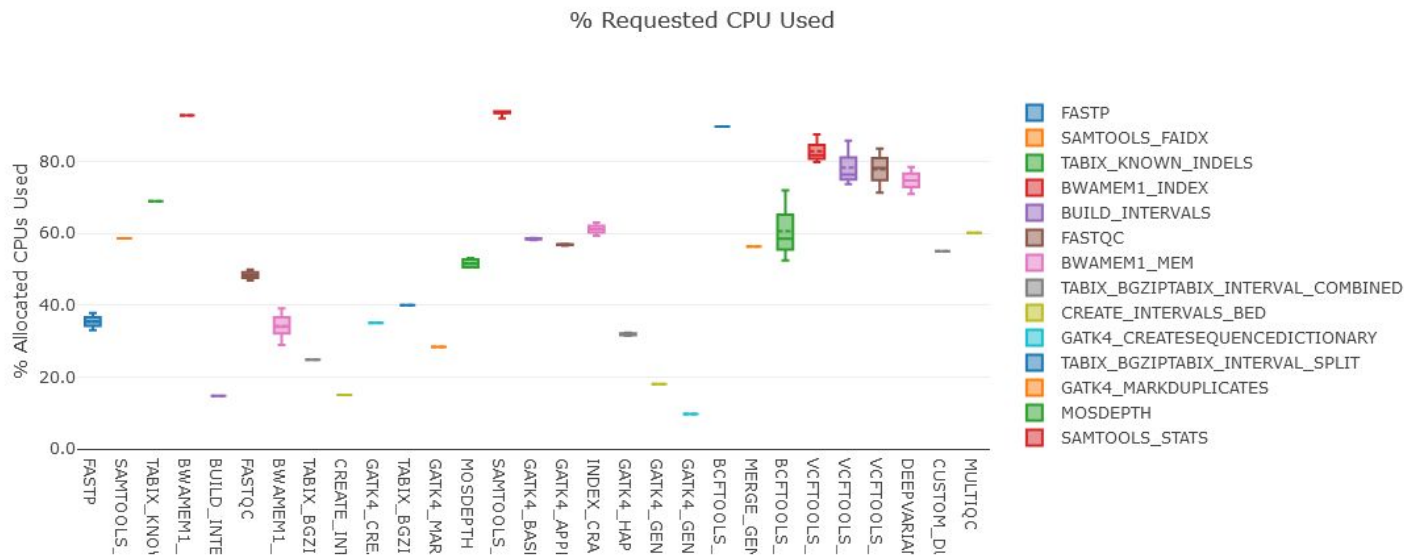
Resource Usage

These plots give an overview of the distribution of resource usage for each process.

CPU

Raw Usage

% Allocated





Exercice 7

