Development of a workflow for SNP detection in grapevine species: MAPHiTS.

MAPHiTS:* *Mappping Analysis Pipeline for High-Throughput Sequences

ISMB / ECCB 2011 – Workshop 6

ALIMENTATION
AGRICULTURE
ENVIRONNEMENT

Overview

I. Background and objectives of the pipeline

II. Existing Tools

III. MAPHiTS Development Tools

IV. Integration of tools in Galaxy

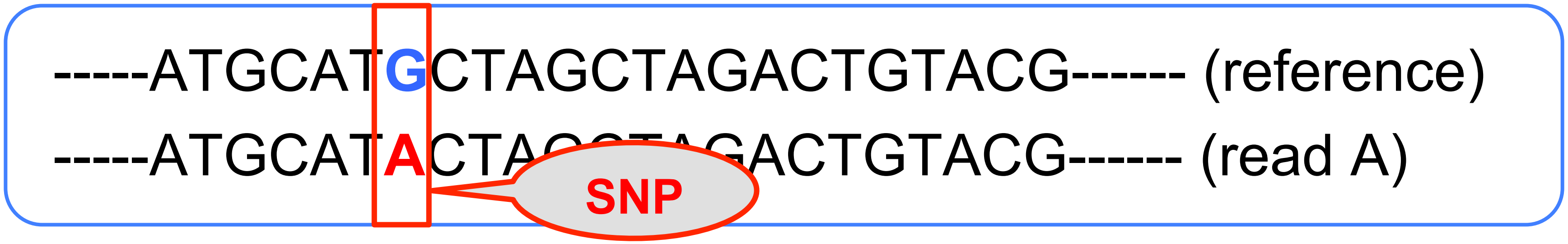
V. Preliminary Results

VI. Perspectives

I. Background and objectives of the pipeline

I. Background and objectives of the pipeline

A **SNP** (Single-Nucleotide Polymorphism) is a DNA sequence variation. SNPs are used to detect complex traits such as diseases restistance or agronomical performance.



-----ATGCATGCTAGCTAGACTGTACG------ (reference)

-----ATGCATACTAGCTAGACTGTACG------ (read A)

URGI team develops a **pipeline** *(MAPHiTS)* for **SNPs detection** from short reads. It's fully integrated in **Galaxy**.

Users : 50% biologists / 50% bioinformaticians

I. Background and objectives of the pipeline

- **Objectives:**

  Detect a set of SNPs between various species of Grape after mapping short reads against a reference genome.

- **Data:**
  - Project 1 : 6 species
  - Project 2 : 16 species

  Short reads are in paired-ends with 76, 101 or 114 bp *(Illumina GAII)*.

⇨ Other projects are also in progress with others species.

II. Existing Tools

II. Existing Tools

- **FASTX-Toolkit:** tools for FASTA / FASTQ files preprocessing.

- **BWA / Bowtie:** mapping softwares, particularly suitable for short reads alignement (in paired-ends or single-ends) against one reference genome (Burrows – Wheeler Alignement tool).

- **SAMtools:** toolkit for working on the output SAM file (BWA, Bowtie, …).

- **VarScan:** software used to filter SNPs and small indels by:
  - coverage
  - number of variant
  - base quality
  - variant allele frequency
  - pValue

III. MAPHiTS Development Tools

III. MAPHiTS Development Tools

- **Optimization tools:**
  - BWA in parallel
  - SAM-to-BAM in parallel

Time Saving: 10x average

Exemple:

- Before: 11 -12 hours
- Now: 1 - 2 hours

- **Preprocessing tools:**
  - Remove duplicated short-reads
  - Remove short reads not in paired-ends
  - Remove short reads > ‘N’%
  - Remove informations in each FASTA file header

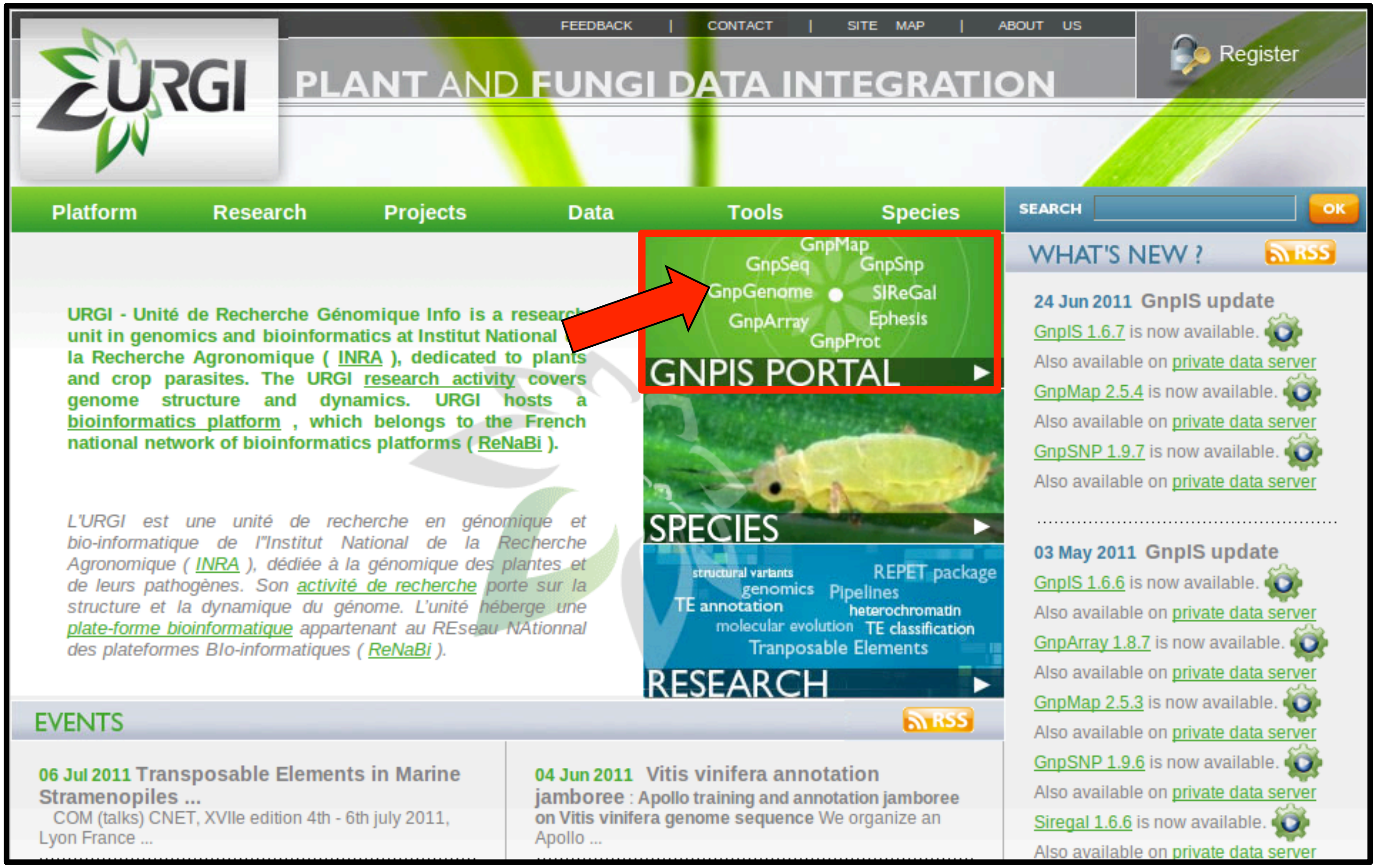
III. MAPHiTS Development Tools

- **Postprocessing tools:**
  - Count multiple hits from the results of BWA
  - Extract short reads from SAM file
  - Extract SNPs with flanks 5’ and 3’
  - Keep SNPs without other SNPs in an interval
  - Keep SNPs without ‘N’ in an interval
  - Remove sequences > ‘N’ % or ‘GC’ %
  - VarScan compare (intersection, merge or unique)
  - VarScan to Gff3

IV. Integration of tools in Galaxy

IV. Integration of tools in Galaxy

http://urgi.versailles.inra.fr/



URGI - Unité de Recherche Génomique Info is a researc unit in genomics and bioinformatics at Institut National la Recherche Agronomique (INRA), dedicated to plants and crop parasites. The URGI research activity covers genome structure and dynamics. URGI hosts a bioinformatics platform , which belongs to the French national network of bioinformatics platforms (ReNaBi).

L'URGI est une unité de recherche en génomique et bio-informatique de l'"Institut National de la Recherche Agronomique (INRA), dédiée à la génomique des plantes et de leurs pathogènes. Son activité de recherche porte sur la structure et la dynamique du génome. L'unité héberge une plate-forme bioinformatique appartenant au REseau NAtionnal des plateformes BIo-informatiques (ReNaBi).

24 Jun 2011 GnpIS update

GnpIS 1.6.7 is now available.
Also available on private data server
GnpMap 2.5.4 is now available.
Also available on private data server
GnpSNP 1.9.7 is now available.
Also available on private data server

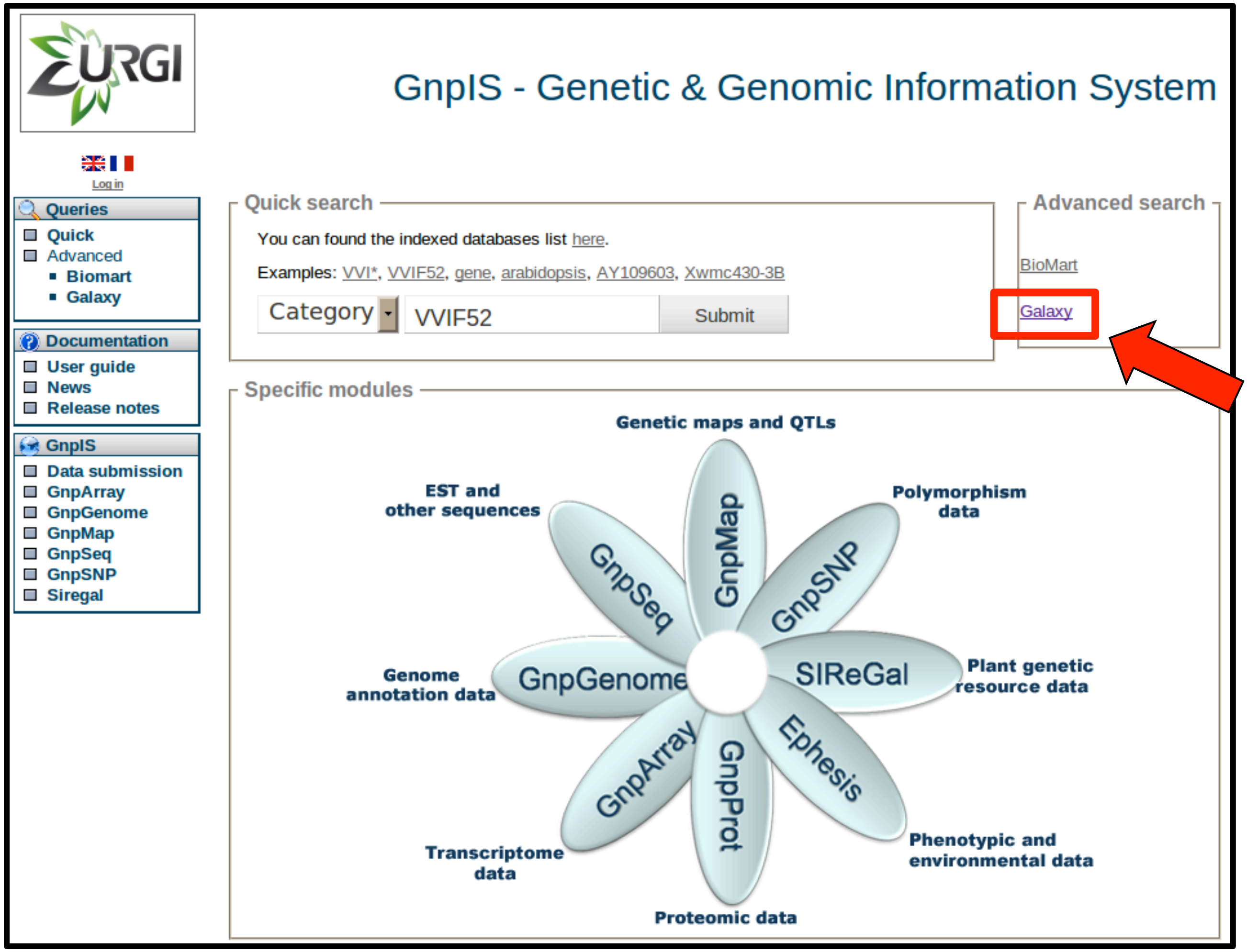
03 May 2011 GnpIS update

GnpIS 1.6.6 is now available.
Also available on private data server
GnpArray 1.8.7 is now available.
Also available on private data server
GnpMap 2.5.3 is now available.
Also available on private data server
GnpSNP 1.9.6 is now available.
Also available on private data server
Siregal 1.6.6 is now available.
Also available on private data server

06 Jul 2011 Transposable Elements in Marine Stramenopiles ...
COM (talks) CNET, XVIIe edition 4th - 6th july 2011, Lyon France ...

04 Jun 2011 Vitis vinifera annotation jamboree : **Apollo training and annotation jamboree on Vitis vinifera genome sequence** We organize an Apollo ...

IV. Integration of tools in Galaxy



GnpIS - Genetic & Genomic Information System

Log in

Queries
- □ Quick
- □ Advanced
  - ▪ Biomart
  - ▪ Galaxy

Documentation
- □ User guide
- □ News
- □ Release notes

GnpIS
- □ Data submission
- □ GnpArray
- □ GnpGenome
- □ GnpMap
- □ GnpSeq
- □ GnpSNP
- □ Siregal

Quick search

You can found the indexed databases list here.

Examples: VVI*, VVIF52, gene, arabidopsis, AY109603, Xwmc430-3B

Category: VVIF52 [Submit]

Advanced search

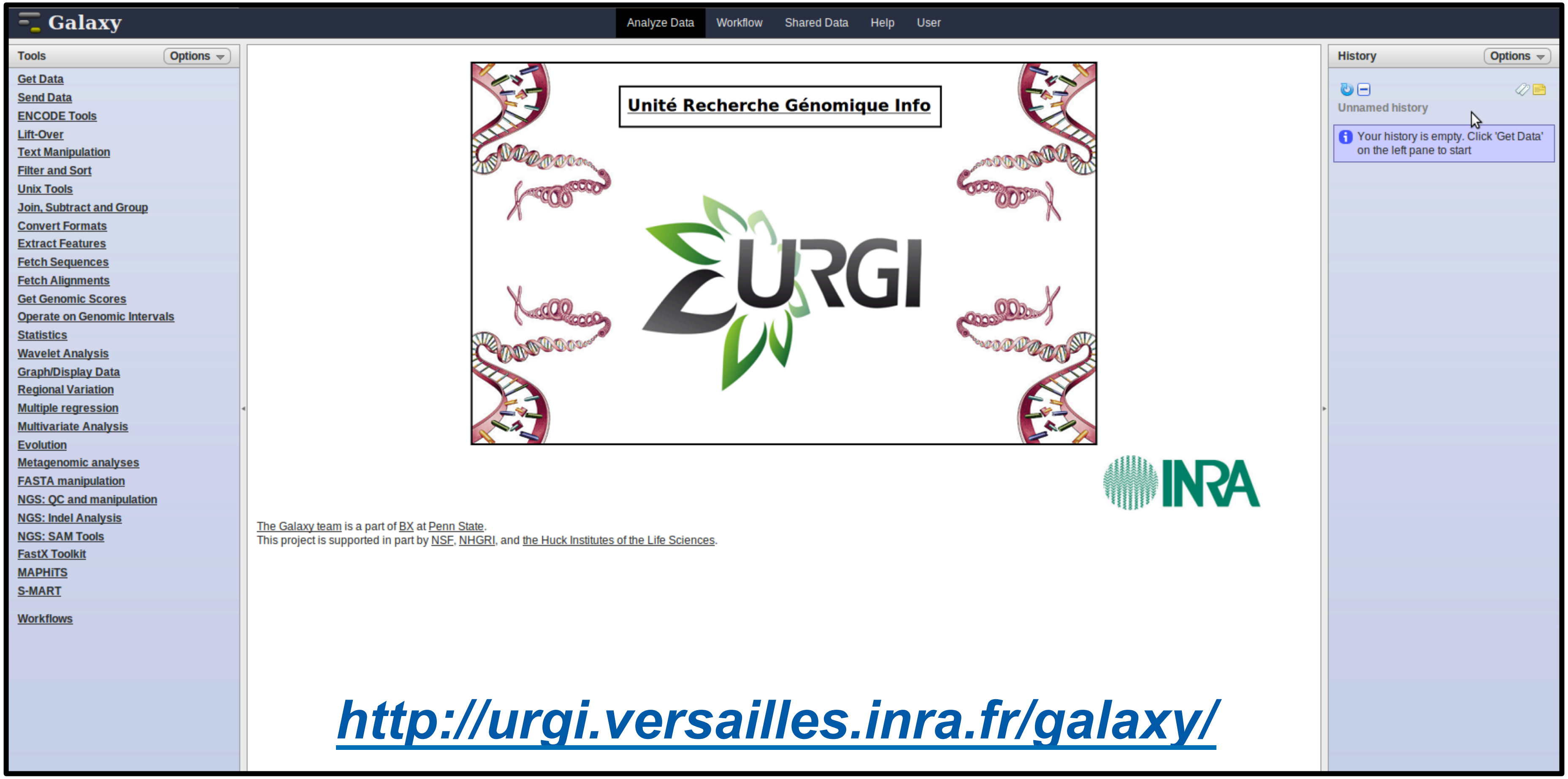
BioMart

Galaxy

Specific modules

Galaxy

IV. Integration of tools in Galaxy

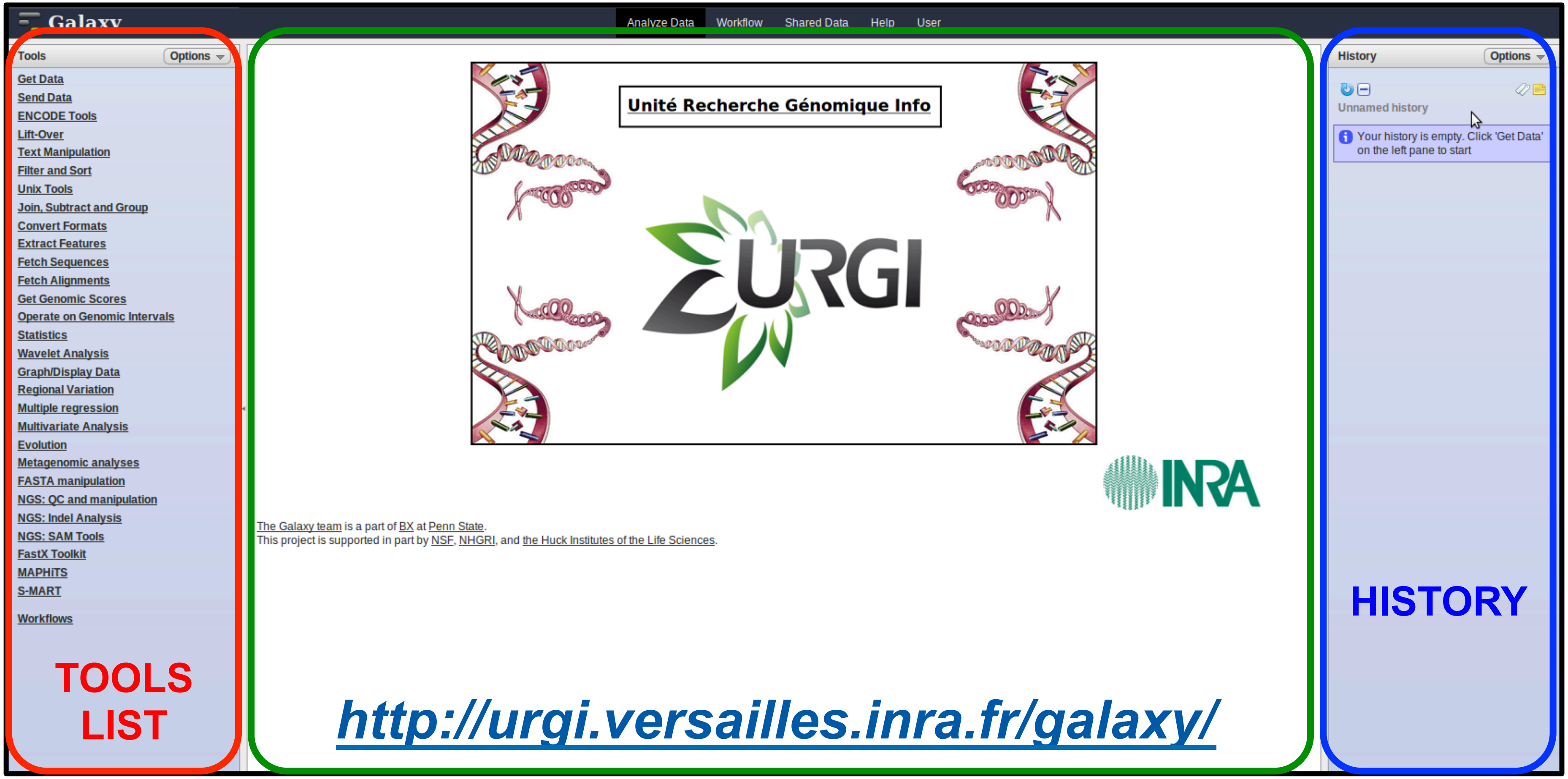


Your history is empty. Click 'Get Data' on the left pane to start

The Galaxy team is a part of BX at Penn State.
This project is supported in part by NSF, NHGRI, and the Huck Institutes of the Life Sciences.

http://urgi.versailles.inra.fr/galaxy/

IV. Integration of tools in Galaxy



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http://urgi.versailles.inra.fr/galaxy/

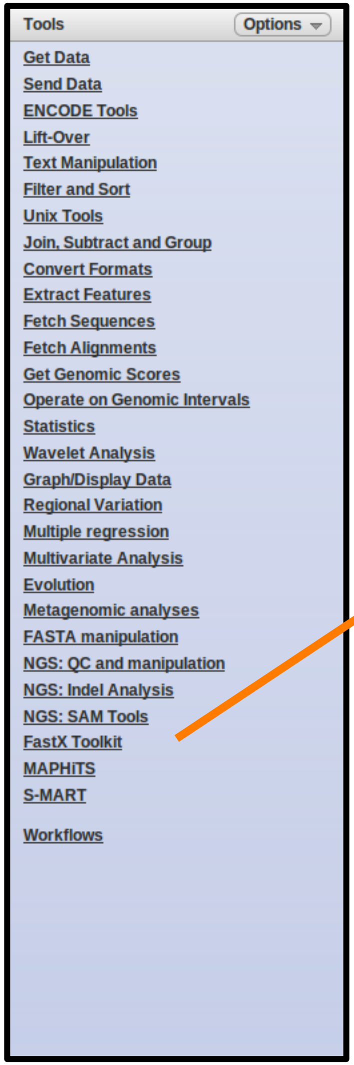
IV.1. Installation of URGI Galaxy

Galaxy is installed on URGI cluster with:

❑ CPU:	**704** (Intel Xeon)
❑ RAM max:	**96 Gb** per job
❑ Storage:	**60 Tb**

Using Sun Grid Engine (for job managment) and a PostgreSQL Database (for Galaxy).

IV.2. New Integrated tools

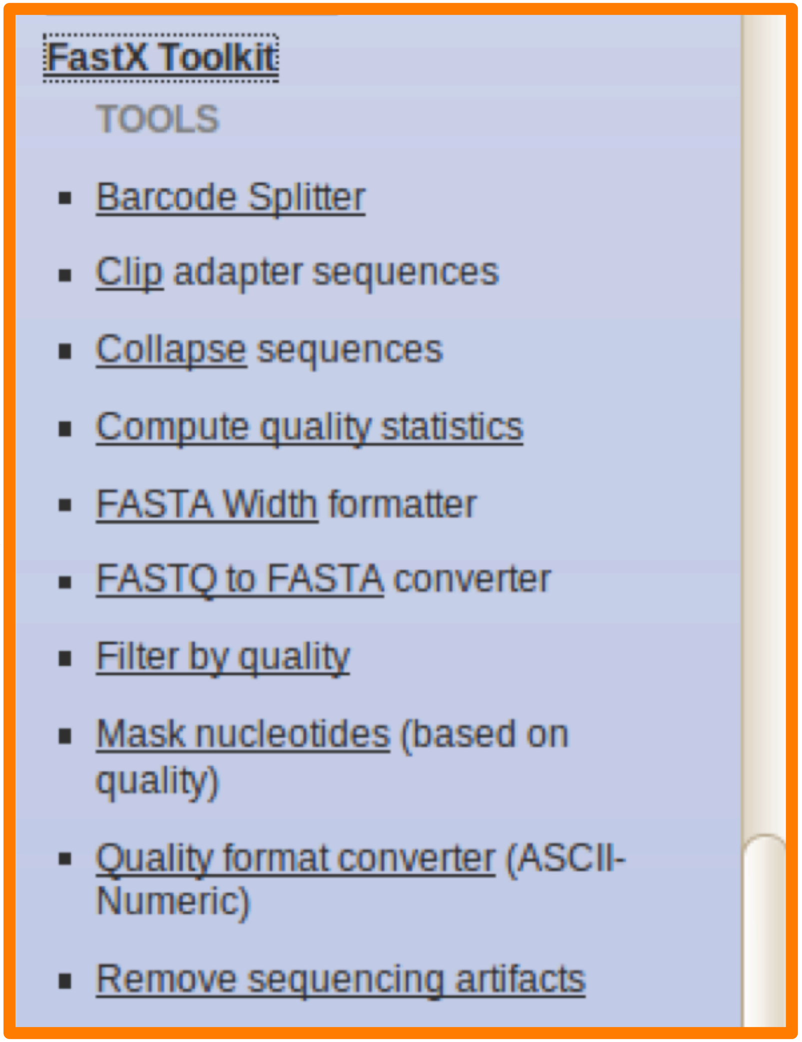


Tools Options

- Get Data
- Send Data
- ENCODE Tools
- Lift-Over
- Text Manipulation
- Filter and Sort
- Unix Tools
- Join, Subtract and Group
- Convert Formats
- Extract Features
- Fetch Sequences
- Fetch Alignments
- Get Genomic Scores
- Operate on Genomic Intervals
- Statistics
- Wavelet Analysis
- Graph/Display Data
- Regional Variation
- Multiple regression
- Multivariate Analysis
- Evolution
- Metagenomic analyses
- FASTA manipulation
- NGS: QC and manipulation
- NGS: Indel Analysis
- NGS: SAM Tools
- FastX Toolkit
- MAPHiTS
- S-MART

Workflows

FASTX-Toolkit

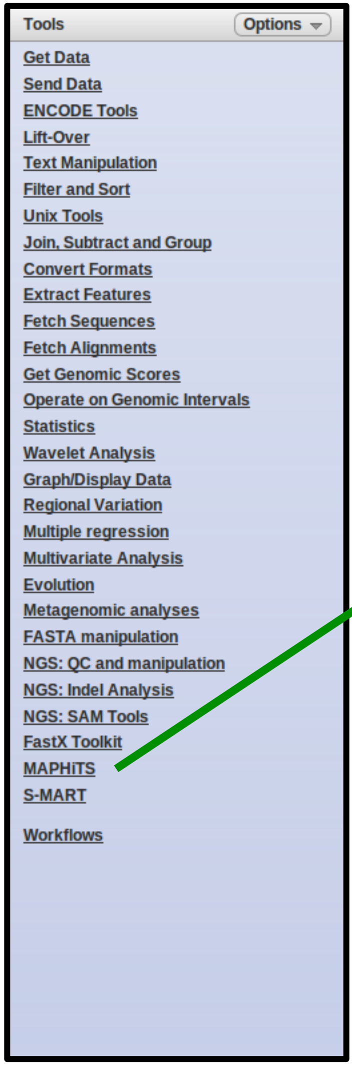


FastX Toolkit

TOOLS

- Barcode Splitter
- Clip adapter sequences
- Collapse sequences
- Compute quality statistics
- FASTA Width formatter
- FASTQ to FASTA converter
- Filter by quality
- Mask nucleotides (based on quality)
- Quality format converter (ASCII-Numeric)
- Remove sequencing artifacts

IV.2. New URGI Integrated tools



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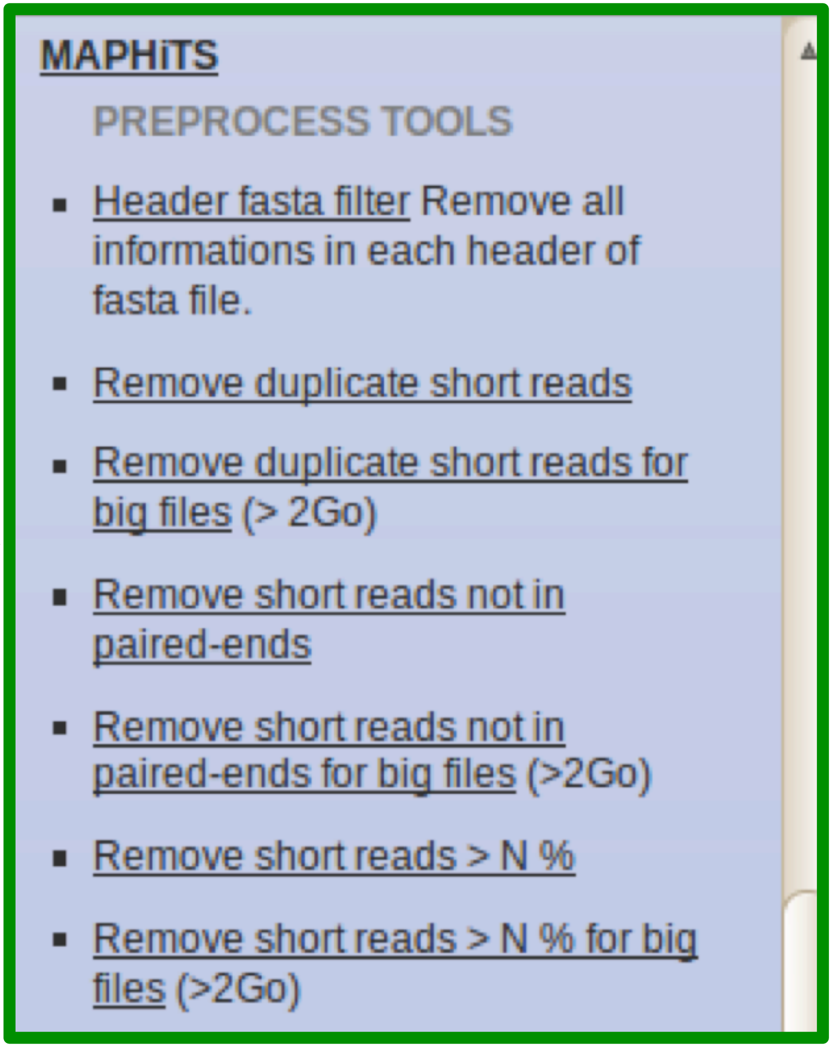
- MAPHiTS
- S-MART

Workflows

MAPHiTS

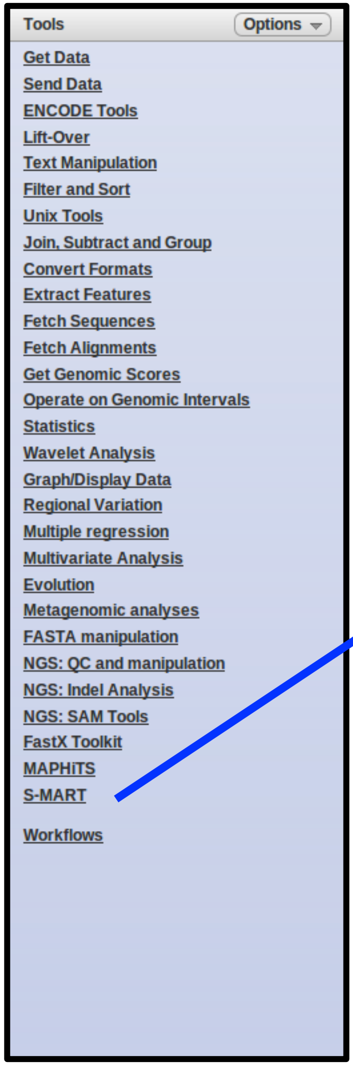


MAPHiTS

PREPROCESS TOOLS

- Header fasta filter Remove all informations in each header of fasta file.
- Remove duplicate short reads
- Remove duplicate short reads for big files (> 2Go)
- Remove short reads not in paired-ends
- Remove short reads not in paired-ends for big files (>2Go)
- Remove short reads > N %
- Remove short reads > N % for big files (>2Go)

IV.2. New Others URGI Integrated tools



Tools Options

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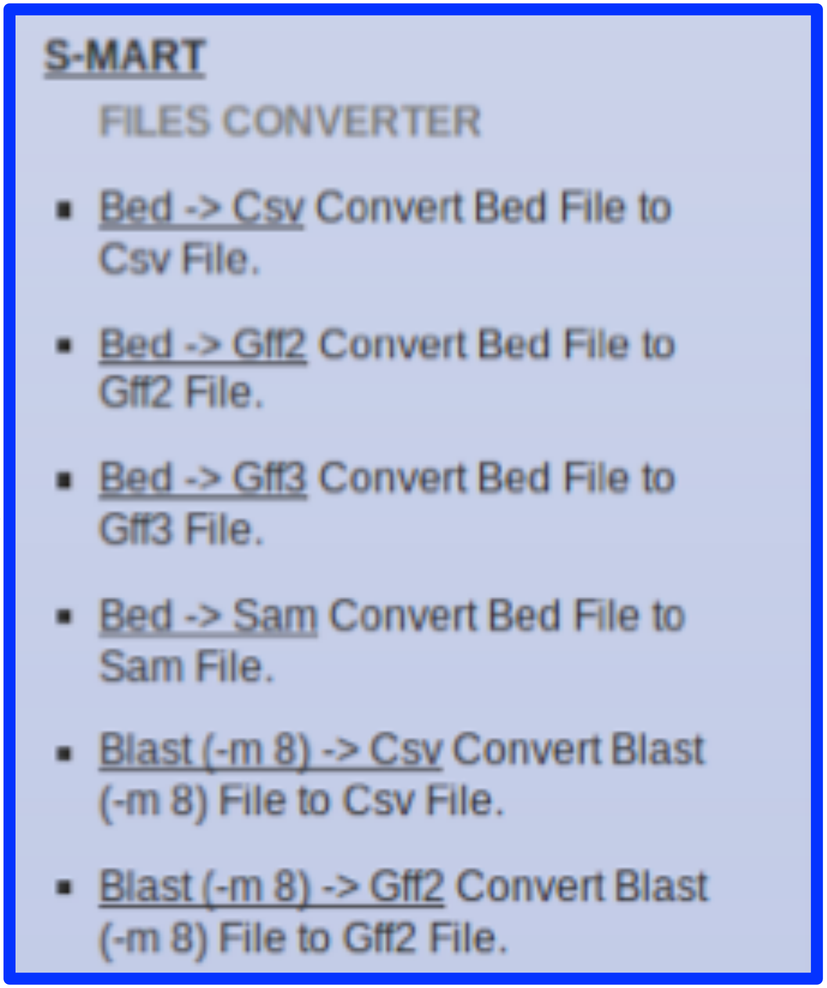
- MAPHiTS
- S-MART

Workflows

S-MART



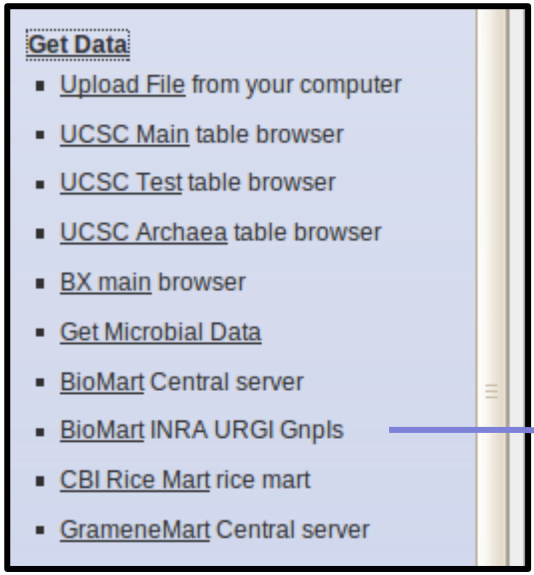
S-MART

FILES CONVERTER

- Bed -> Csv Convert Bed File to Csv File.
- Bed -> Gff2 Convert Bed File to Gff2 File.
- Bed -> Gff3 Convert Bed File to Gff3 File.
- Bed -> Sam Convert Bed File to Sam File.
- Blast (-m 8) -> Csv Convert Blast (-m 8) File to Csv File.
- Blast (-m 8) -> Gff2 Convert Blast (-m 8) File to Gff2 File.

IV.2. New Others URGI Integrated tools

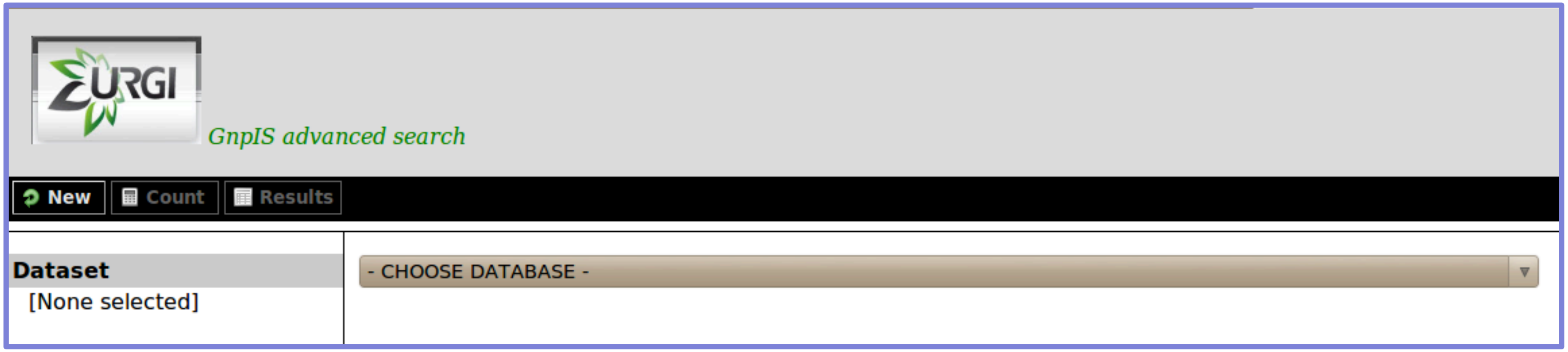
Access to URGI Information System via **BioMart** software



Get Data

- Upload File from your computer
- UCSC Main table browser
- UCSC Test table browser
- UCSC Archaea table browser
- BX main browser
- Get Microbial Data
- BioMart Central server
- BioMart INRA URGI Gnpls
- CBI Rice Mart rice mart
- GrameneMart Central server

BioMart URGI Gnpls



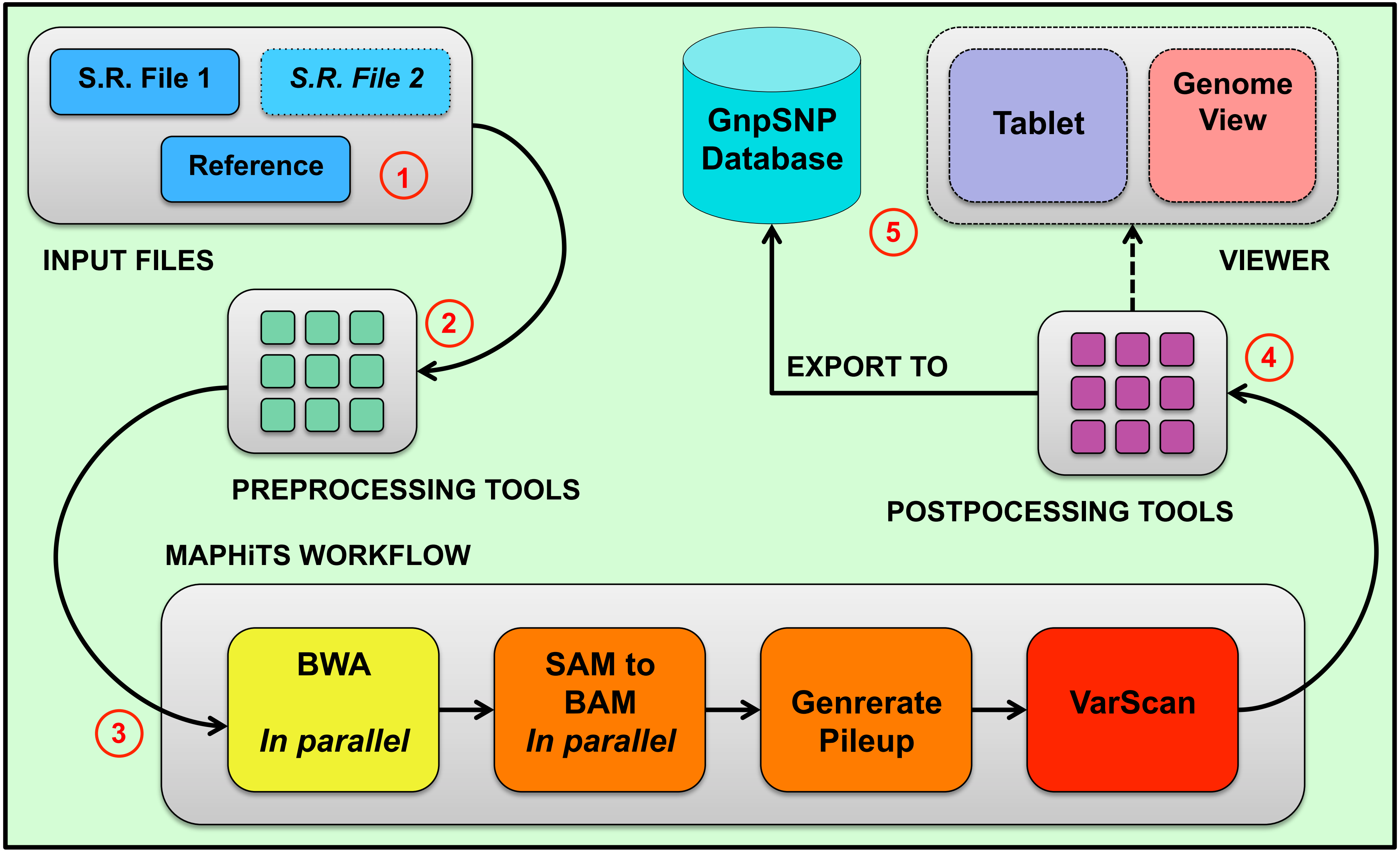
GnpIS advanced search

Dataset

[None selected]

- CHOOSE DATABASE -

IV.3. MAPHiTS: Resume



IV.3. MAPHiTS: Resume

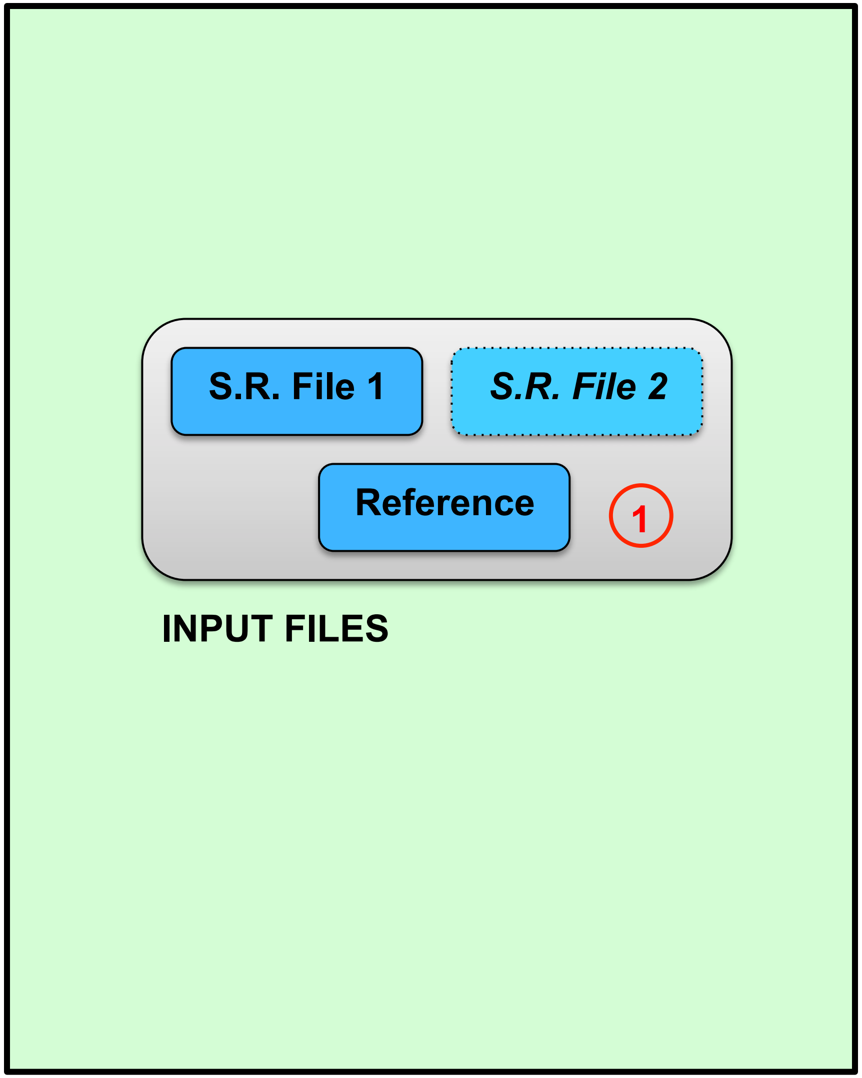
Step 1

Upload your input files:

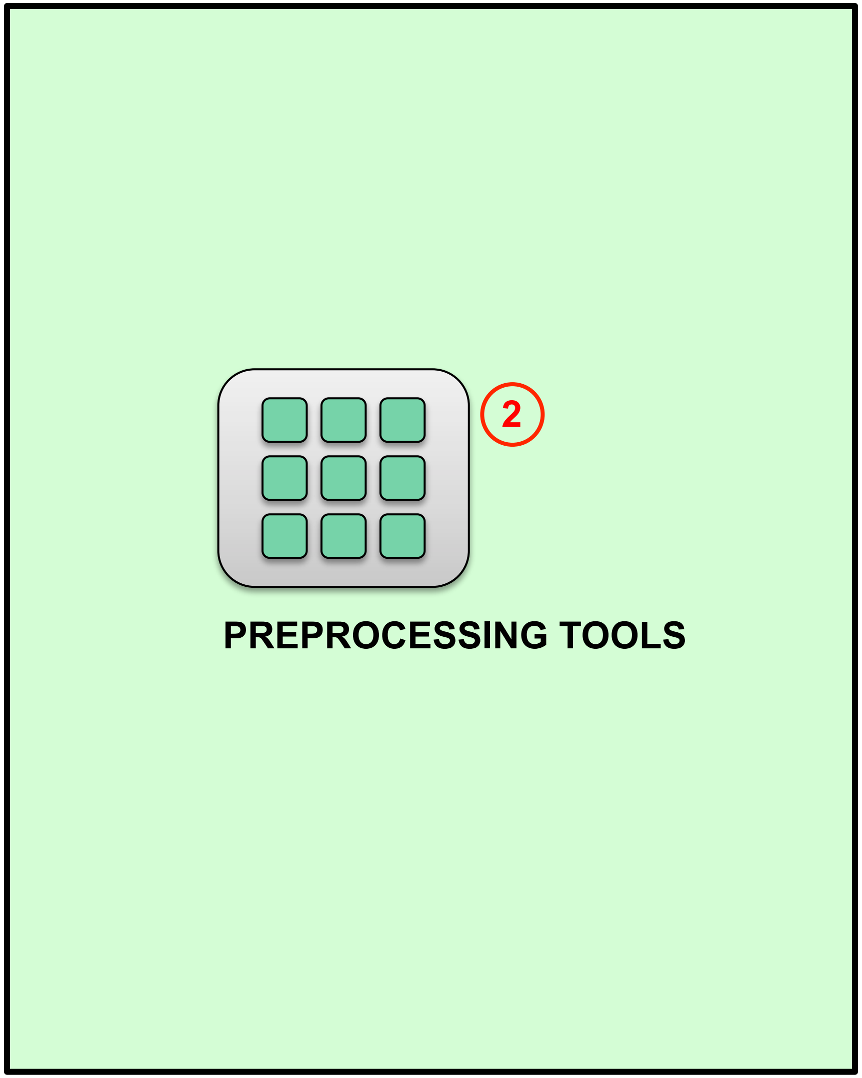
- 1 reference genome (FASTA)
- 1 short reads file if you are in single-ends (FASTA / FASTQ)

OR

- 2 short reads files if you are in paired-ends (FASTA / FASTQ)



IV.3. MAPHiTS: Resume



PREPROCESSING TOOLS

Step 2

You can filter your input files with one or some preprocessing tools.

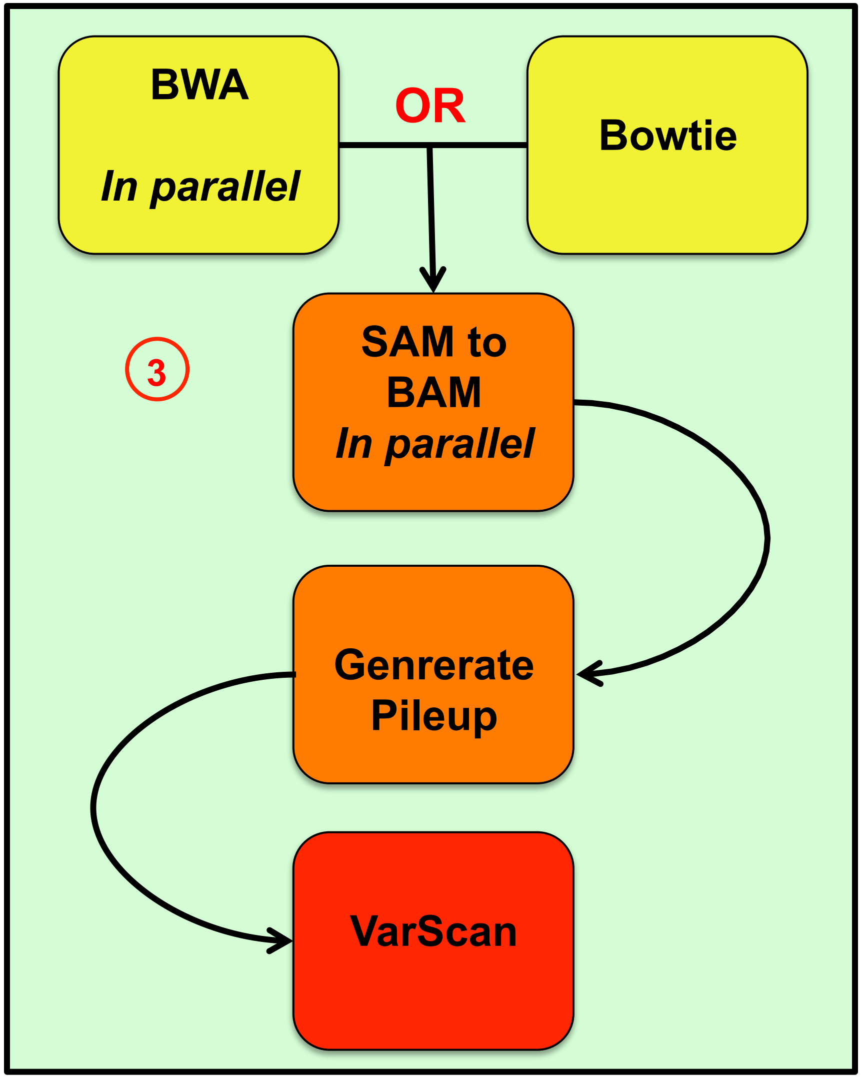
Examples:

- Remove all duplicated short reads
- Trim short reads by quality
- Remove short reads not in paired-ends

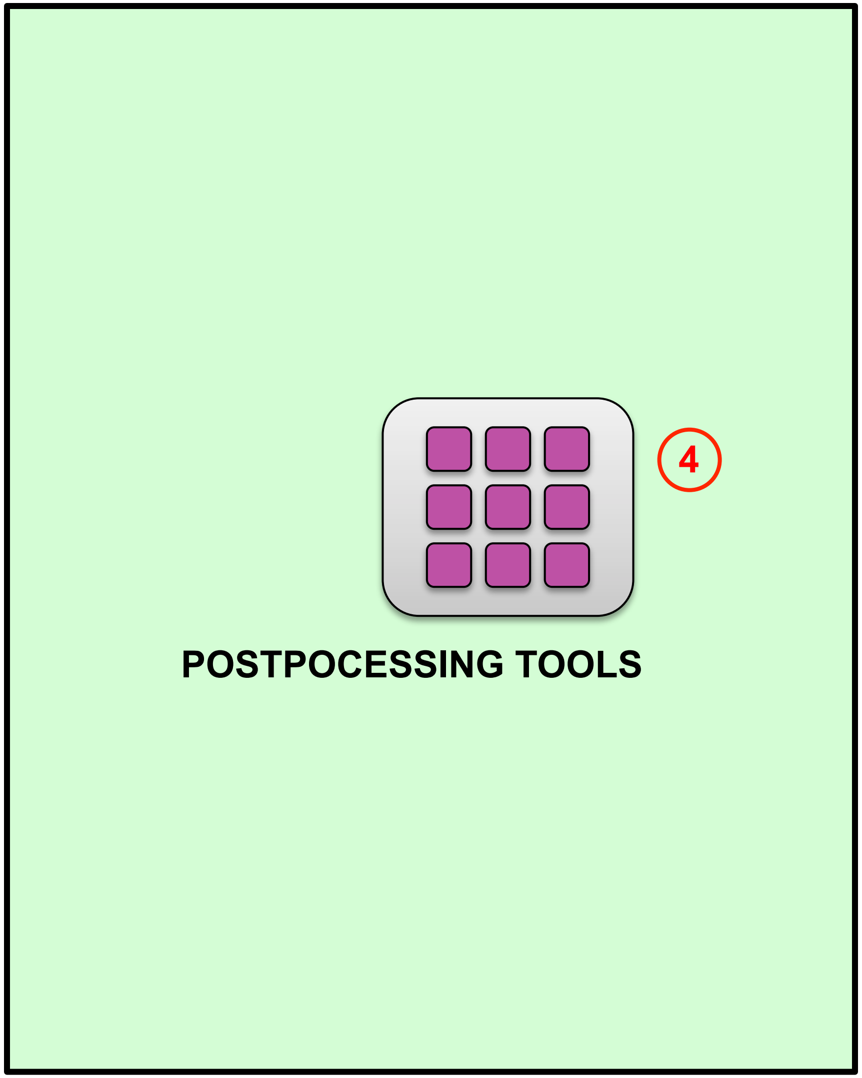
IV.3. MAPHiTS: Resume

Step 3

You have to launch MAPHiTS workflow.



IV.3. MAPHiTS: Resume



POSTPOCESSING TOOLS

Step 4

You can filter your output files with one or some postprocessing tools.

Examples:

- Count multiple hits from the results of BWA
- Extract short reads from SAM file
- VarScan compare

IV.3. MAPHiTS: Resume

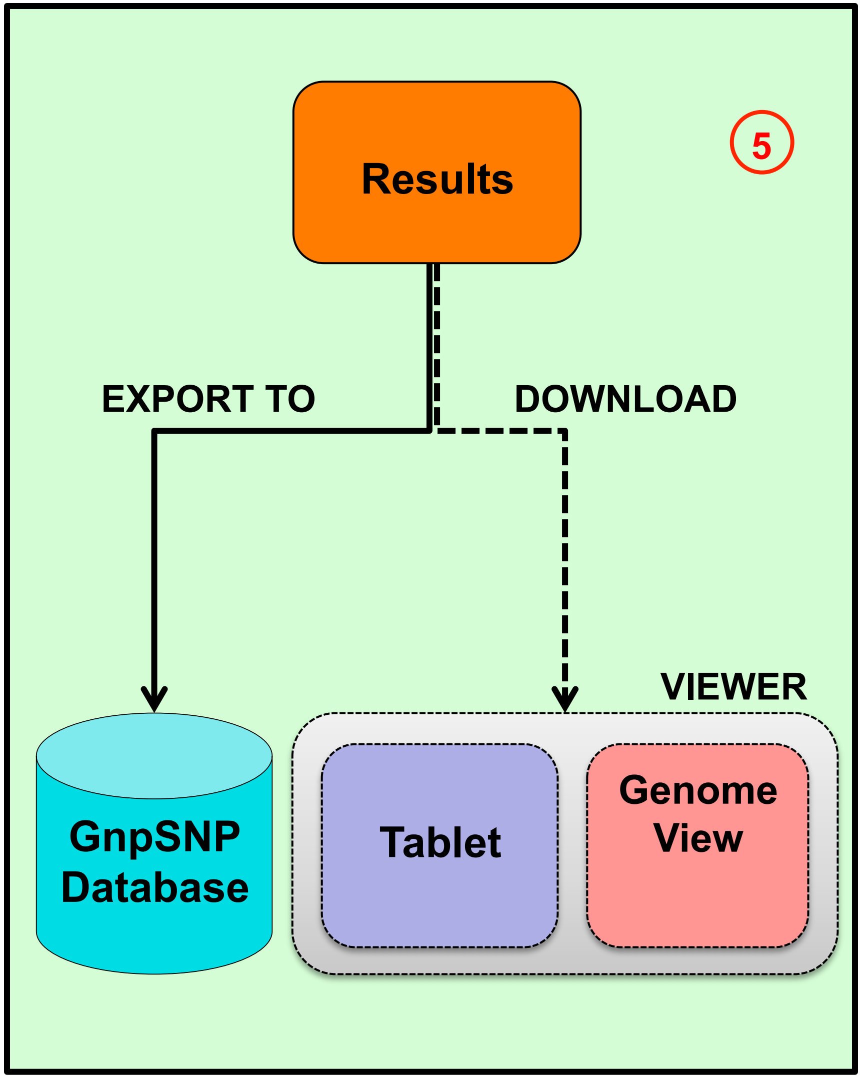
Step 5

Finally, you can :

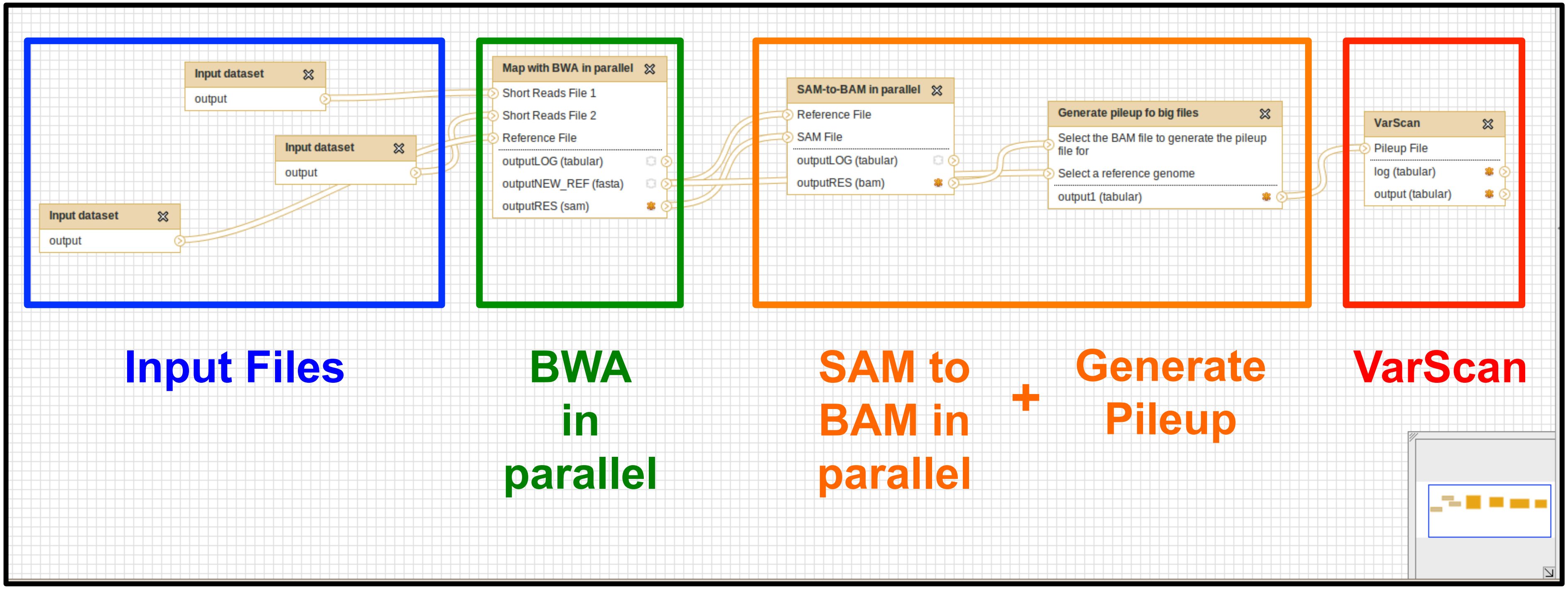
- Export your results to our GnpSNP database

OR / AND

- Download your results and visualize them with your favorite viewer software (Tablet, GenomeView, Gbrowse 2, IGV, …)



IV.3. MAPHiTS: Build



Input Files

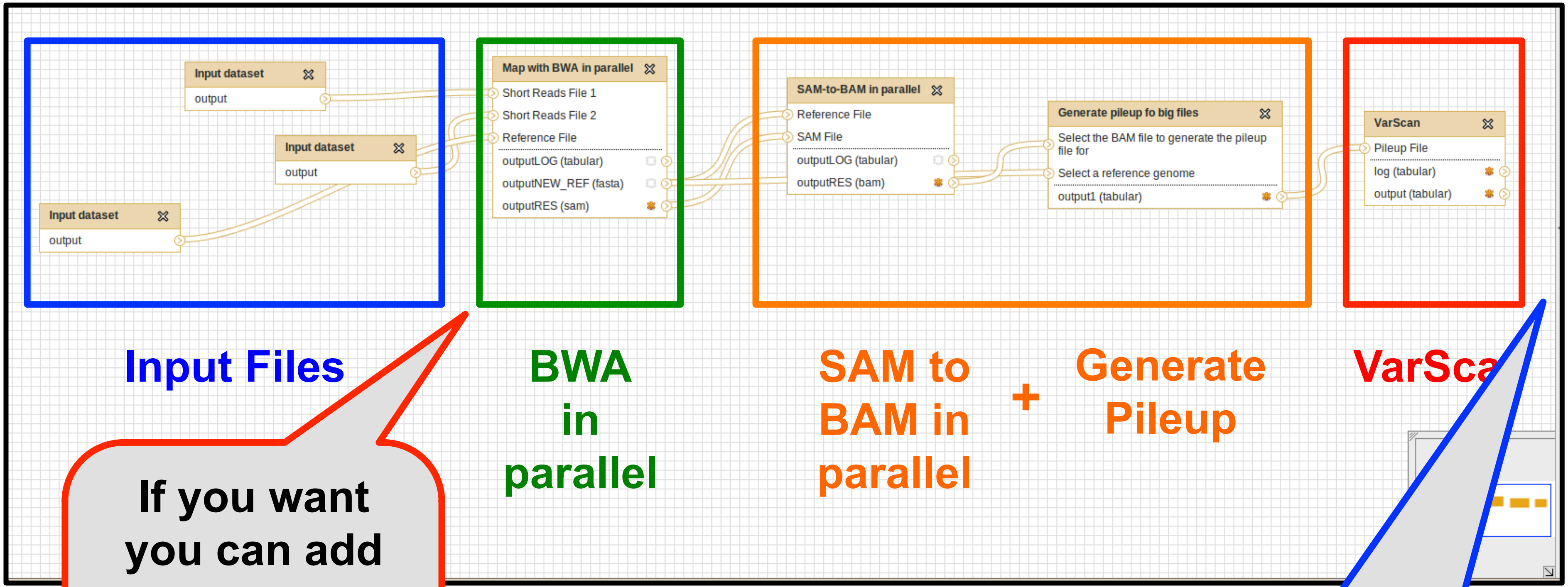
BWA in parallel

SAM to BAM in parallel + Generate Pileup

VarScan

MAPHiTS is build using the graphical interface of Galaxy.

IV.3. MAPHiTS: Build



Input Files

BWA in parallel

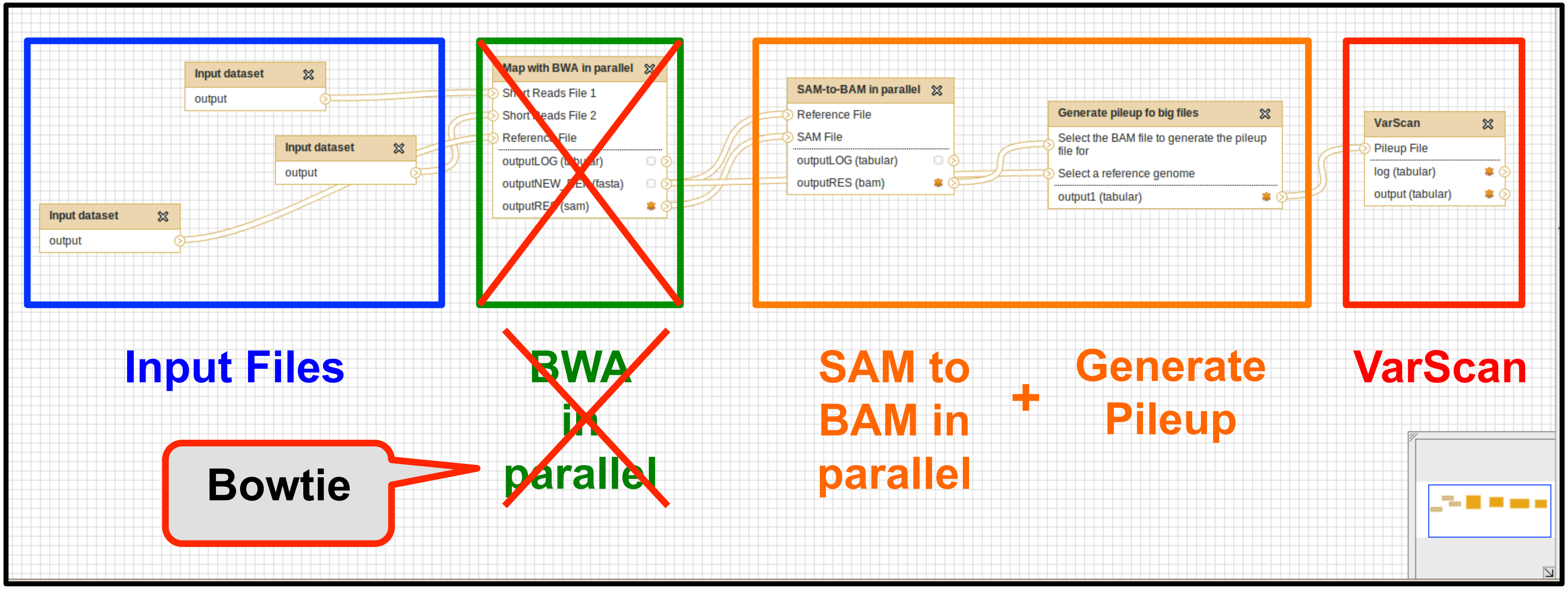
SAM to BAM in parallel + Generate Pileup

VarSca

If you want you can add some preprocessing tools …

… or some postprocessing tools

IV.3. MAPHiTS: Build



Input dataset
output

Input dataset
output

Input dataset
output

~~Map with BWA in parallel~~
~~Short Reads File 1~~
~~Short Reads File 2~~
~~Reference File~~
~~outputLOG (tabular)~~
~~outputNEW_REF (fasta)~~
~~outputRES (sam)~~

SAM-to-BAM in parallel
Reference File
SAM File
outputLOG (tabular)
outputRES (bam)

Generate pileup fo big files
Select the BAM file to generate the pileup file for
Select a reference genome
output1 (tabular)

VarScan
Pileup File
log (tabular)
output (tabular)

Input Files

~~BWA in parallel~~

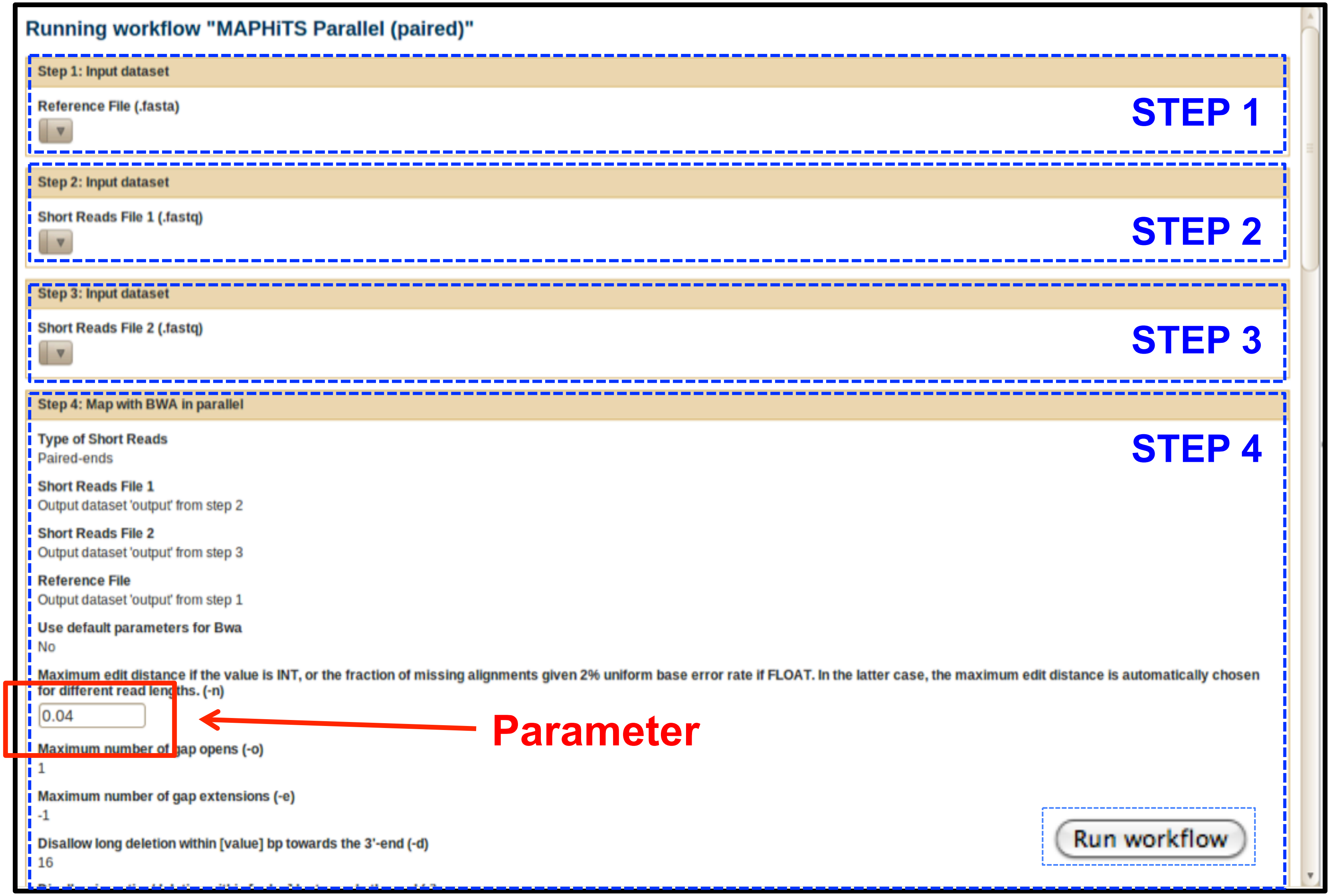
Bowtie

SAM to BAM in parallel + Generate Pileup

VarScan

You can **remove** one tool **and replace** it by an other tool very **quickly**.

IV.3. MAPHiTS: Launch



Running workflow "MAPHiTS Parallel (paired)"

Step 1: Input dataset

Reference File (.fasta)

STEP 1

Step 2: Input dataset

Short Reads File 1 (.fastq)

STEP 2

Step 3: Input dataset

Short Reads File 2 (.fastq)

STEP 3

Step 4: Map with BWA in parallel

STEP 4

Type of Short Reads
Paired-ends

Short Reads File 1
Output dataset 'output' from step 2

Short Reads File 2
Output dataset 'output' from step 3

Reference File
Output dataset 'output' from step 1

Use default parameters for Bwa
No

Maximum edit distance if the value is INT, or the fraction of missing alignments given 2% uniform base error rate if FLOAT. In the latter case, the maximum edit distance is automatically chosen for different read lengths. (-n)
0.04

Parameter

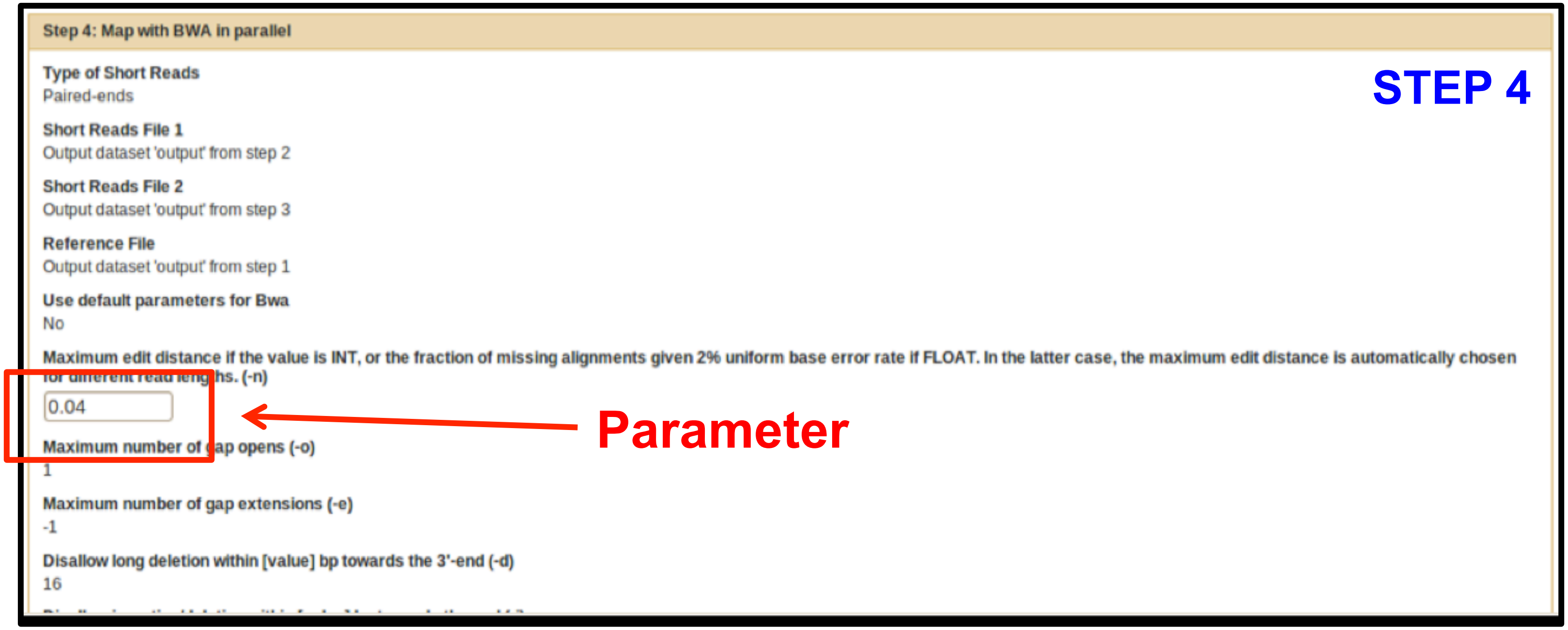
Maximum number of gap opens (-o)
1

Maximum number of gap extensions (-e)
-1

Disallow long deletion within [value] bp towards the 3'-end (-d)
16

IV.3. MAPHiTS: Launch

When I build the workflow, I **can choose** what are the parameters that users **can modify or not**.



Step 4: Map with BWA in parallel

STEP 4

Type of Short Reads
Paired-ends

Short Reads File 1
Output dataset 'output' from step 2

Short Reads File 2
Output dataset 'output' from step 3

Reference File
Output dataset 'output' from step 1

Use default parameters for Bwa
No

Maximum edit distance if the value is INT, or the fraction of missing alignments given 2% uniform base error rate if FLOAT. In the latter case, the maximum edit distance is automatically chosen for different read lengths. (-n)
0.04

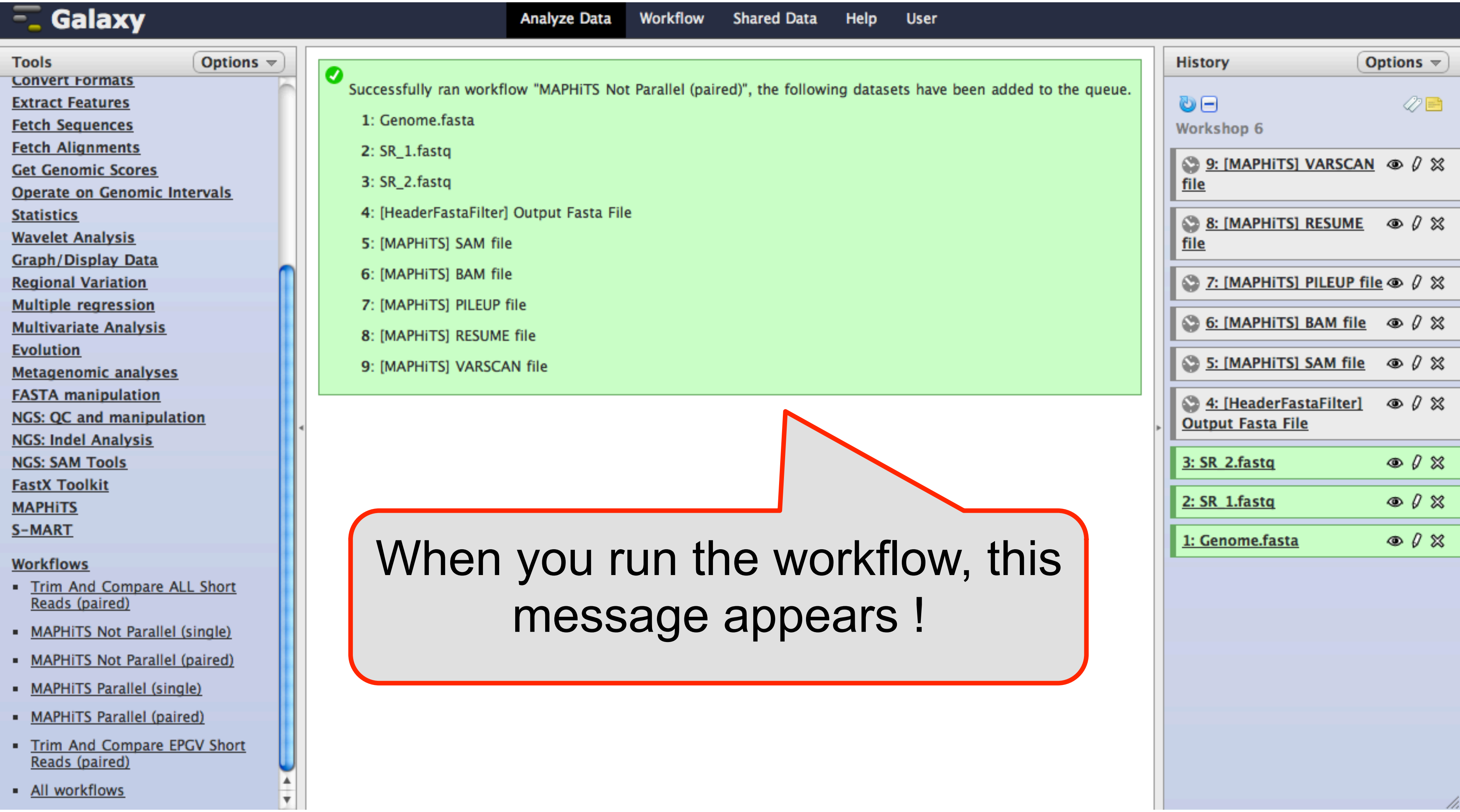
Parameter

Maximum number of gap opens (-o)
1

Maximum number of gap extensions (-e)
-1

Disallow long deletion within [value] bp towards the 3'-end (-d)
16

IV.3. MAPHiTS: Launch



✓ Successfully ran workflow "MAPHiTS Not Parallel (paired)", the following datasets have been added to the queue.

1: Genome.fasta

2: SR_1.fastq

3: SR_2.fastq

4: [HeaderFastaFilter] Output Fasta File

5: [MAPHiTS] SAM file

6: [MAPHiTS] BAM file

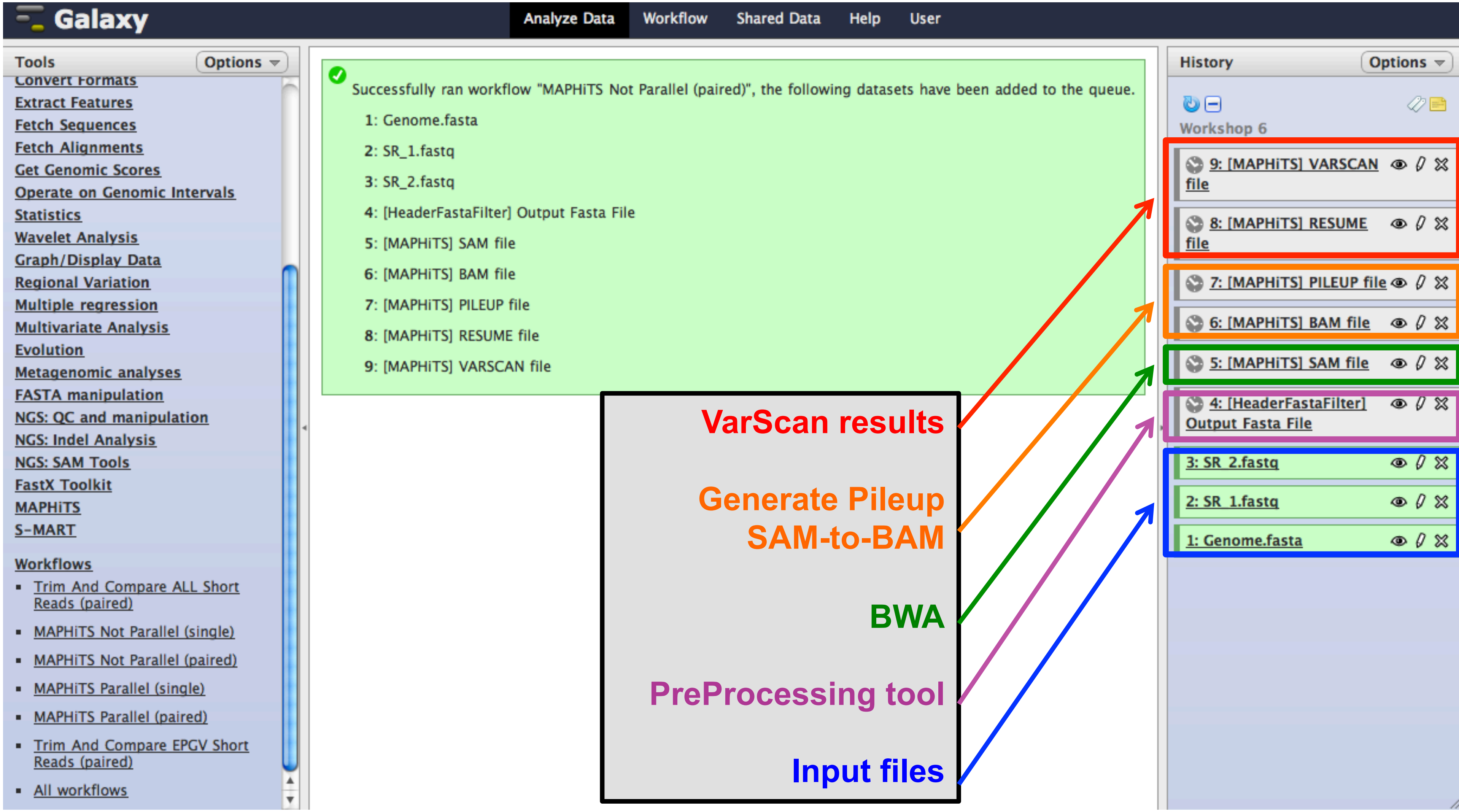
7: [MAPHiTS] PILEUP file

8: [MAPHiTS] RESUME file

9: [MAPHiTS] VARSCAN file

When you run the workflow, this message appears !

IV.3. MAPHiTS: Launch



Successfully ran workflow "MAPHiTS Not Parallel (paired)", the following datasets have been added to the queue.

1: Genome.fasta
2: SR_1.fastq
3: SR_2.fastq
4: [HeaderFastaFilter] Output Fasta File
5: [MAPHiTS] SAM file
6: [MAPHiTS] BAM file
7: [MAPHiTS] PILEUP file
8: [MAPHiTS] RESUME file
9: [MAPHiTS] VARSCAN file

9: [MAPHiTS] VARSCAN file
8: [MAPHiTS] RESUME file
7: [MAPHiTS] PILEUP file
6: [MAPHiTS] BAM file
5: [MAPHiTS] SAM file
4: [HeaderFastaFilter] Output Fasta File
3: SR_2.fastq
2: SR_1.fastq
1: Genome.fasta

VarScan results

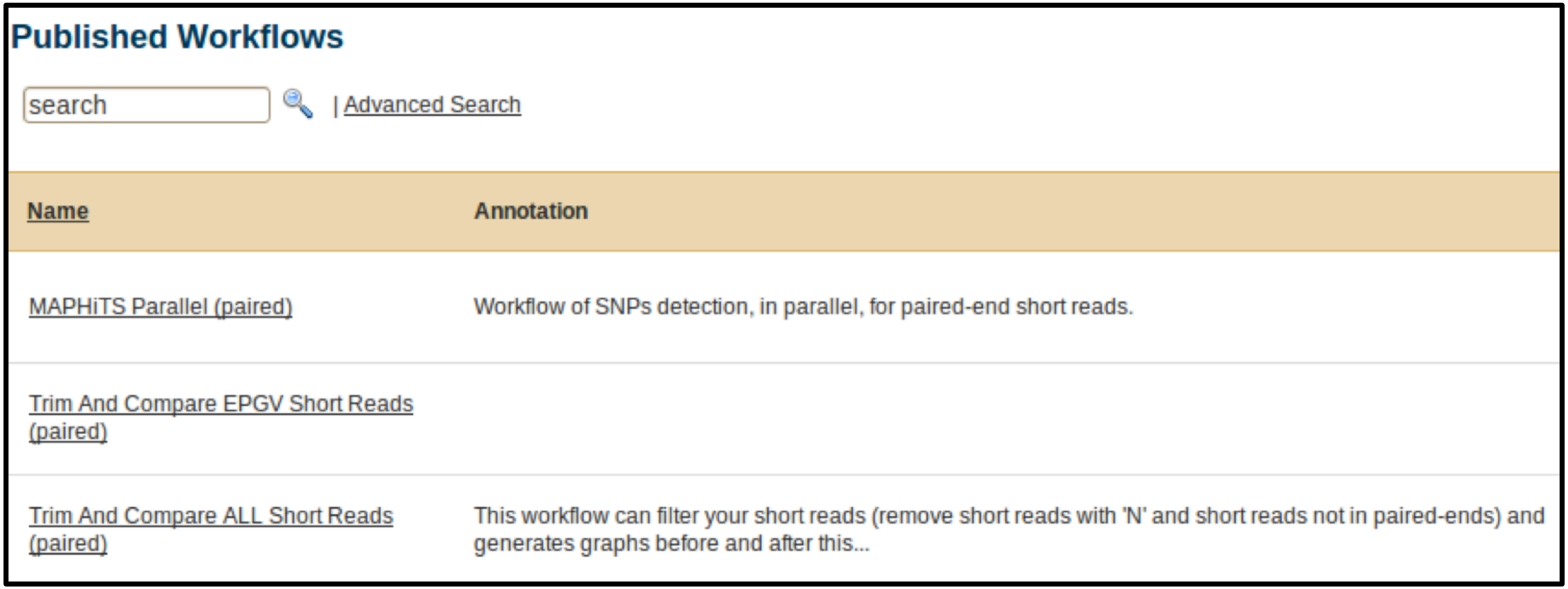
Generate Pileup
SAM-to-BAM

BWA

PreProcessing tool

Input files

IV.4. Shared Workflows / Data



Published Workflows

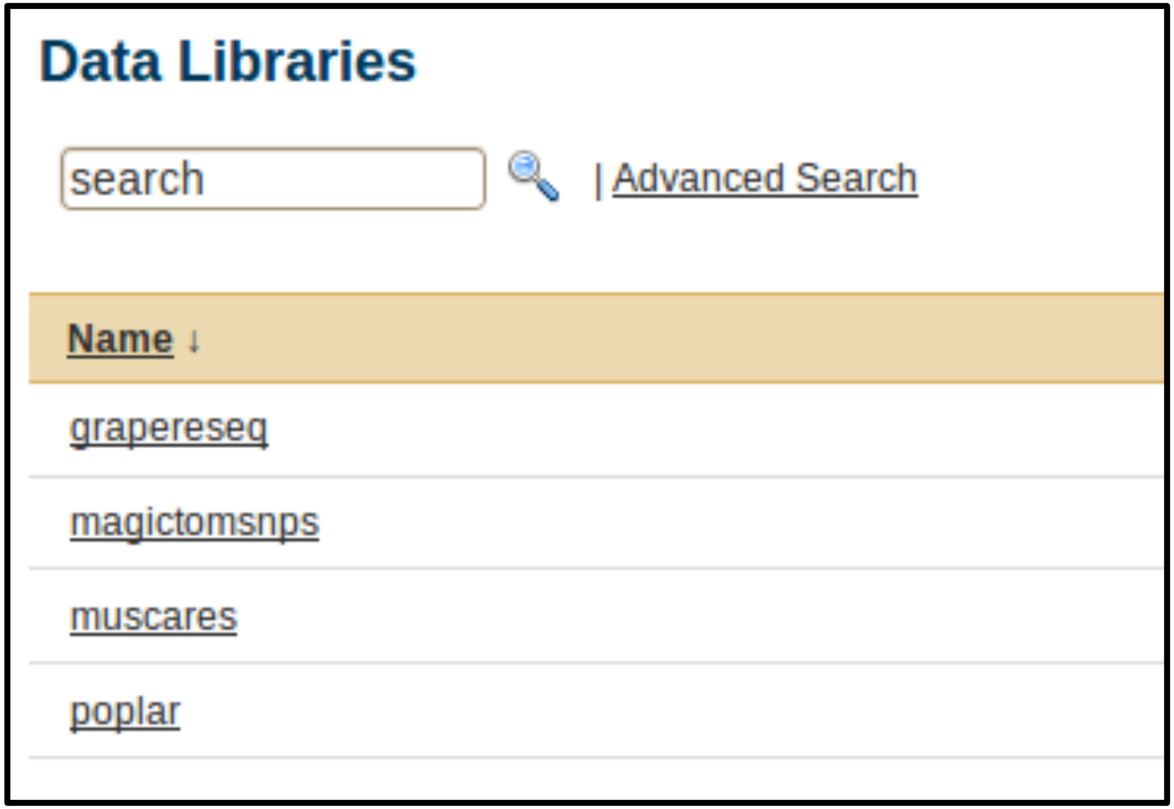
search | Advanced Search

Name	Annotation
MAPHiTS Parallel (paired)	Workflow of SNPs detection, in parallel, for paired-end short reads.
Trim And Compare EPGV Short Reads (paired)	
Trim And Compare ALL Short Reads (paired)	This workflow can filter your short reads (remove short reads with 'N' and short reads not in paired-ends) and generates graphs before and after this...

Some workflows are available for logged users in *'Shared Data'* and *'Published Workflows'* section.

IV.4. Shared Workflows / Data

- In ‘Shared Data’ and ‘Data Libraries’ section, logged users can see 1 directory per Project.
- Users can only see their projects.

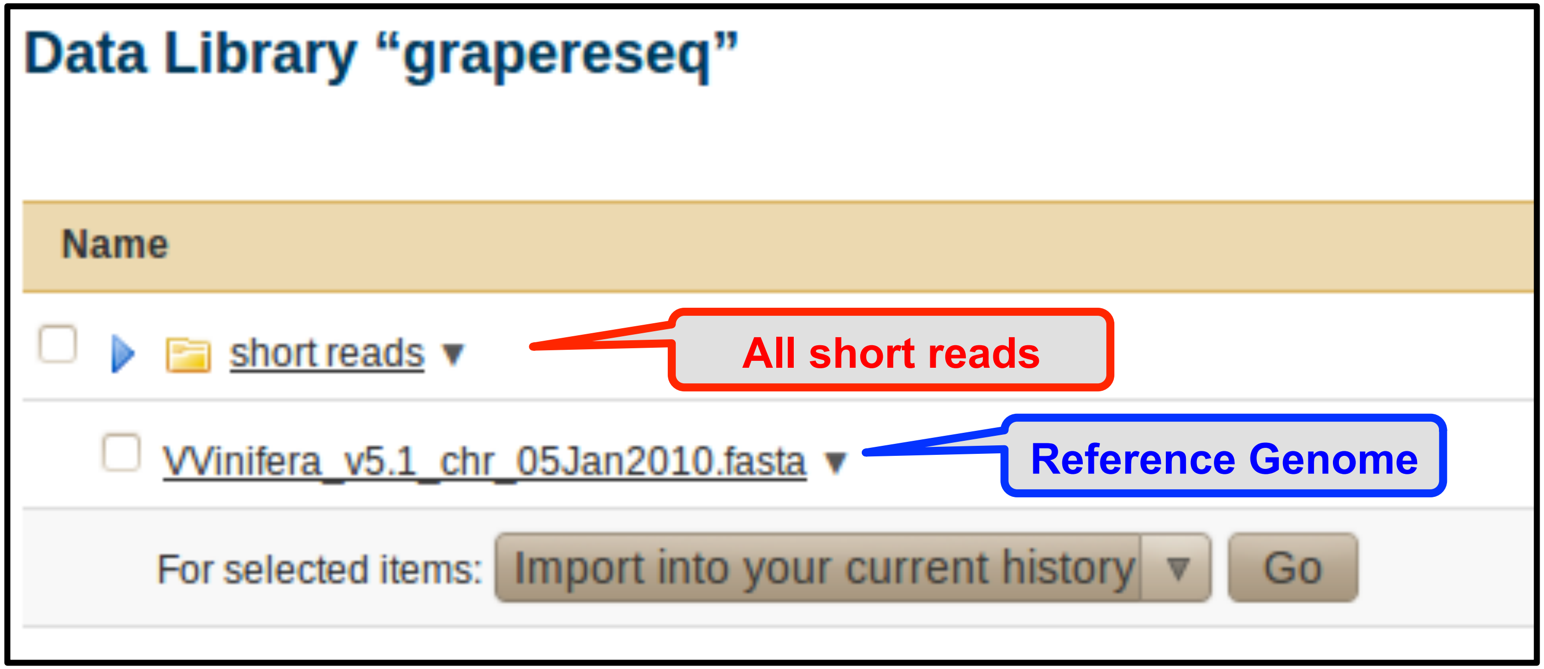


Data Libraries

search | Advanced Search

Name ↓
grapereseq
magictomsnps
muscares
poplar

IV.4. Shared Workflows / Data



Data Library “grapereseq”

Name
□ ▶ short reads ▼
□ VVinifera_v5.1_chr_05Jan2010.fasta ▼

For selected items: Import into your current history ▼ Go

All short reads

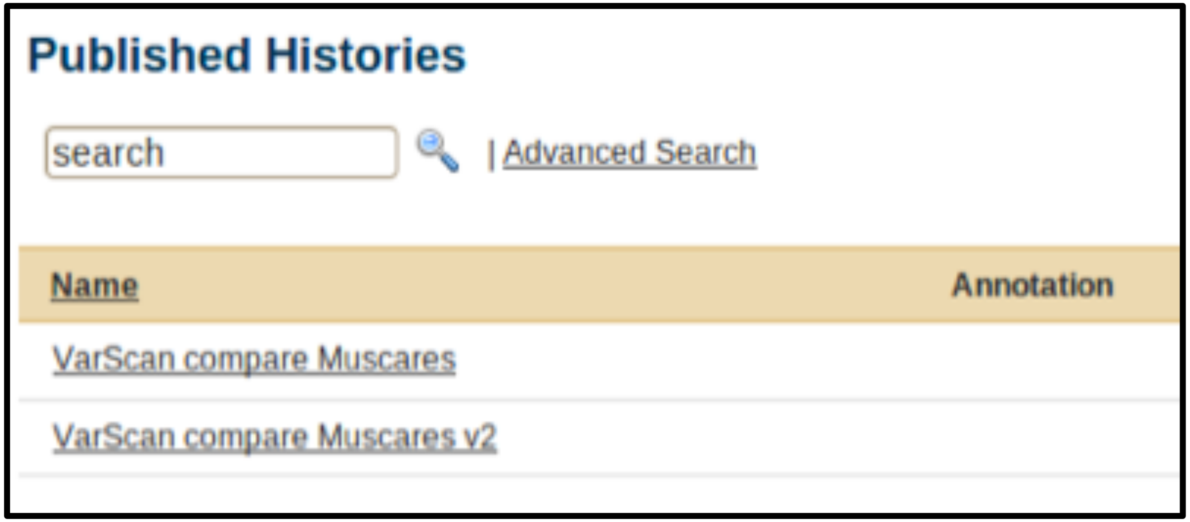
Reference Genome

They can import their data into the history quickly.

➡ Usefull for **NGS** !

IV.5. Shared your History

If a user wants to share its results with other users or a specific user, it's possible !



Published Histories

search | Advanced Search

Name	Annotation
VarScan compare Muscares	
VarScan compare Muscares v2	

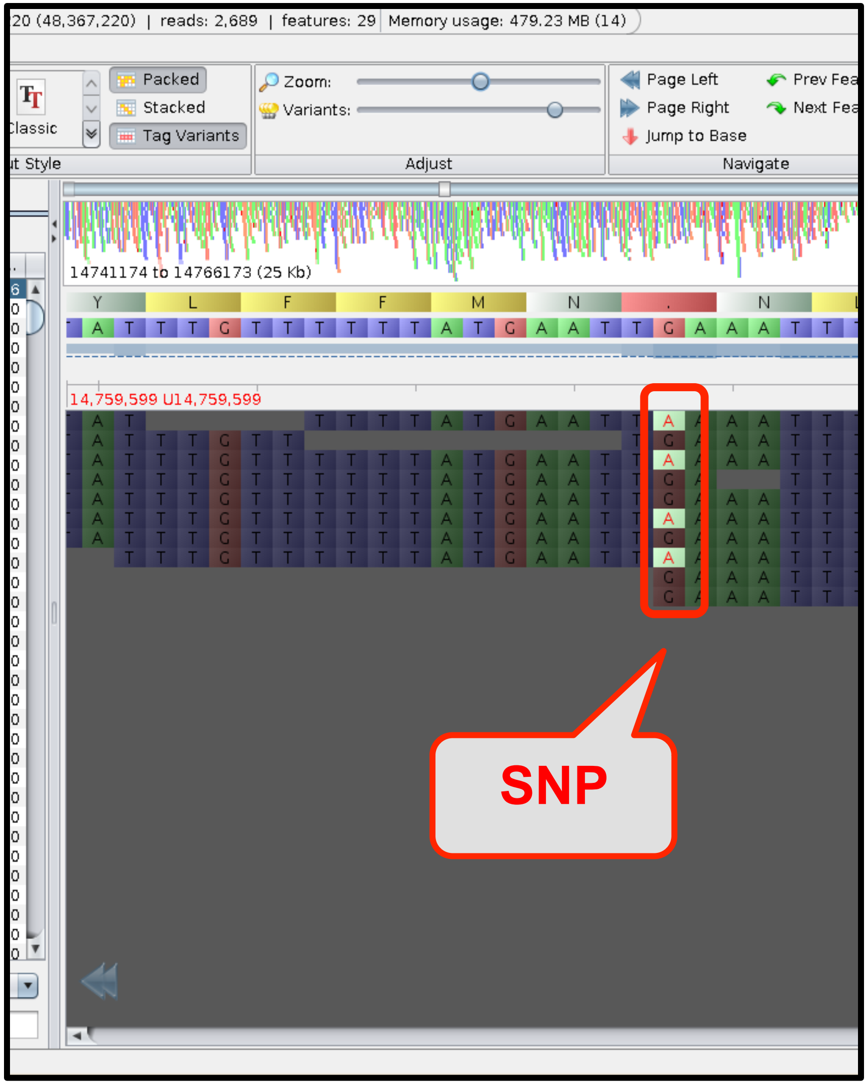
All this histories are in *'Shared Data'* and *'Published Histories'*.

V. Preliminary Results

V. Preliminary Results

We consider that a variant is a SNP if you have:

- **10** short reads in minimum at this position
- **4** variants in minimum
- **30** of mapping quality in minimum
- **30%** of variant allele frequency in minimum
- Pvalue threshold <= **1^{e}10-3**



20 (48,367,220) | reads: 2,689 | features: 29 | Memory usage: 479.23 MB (14)

V. Preliminary Results

	A	B	C
S.R.	**71 Millions**	**70 Millions**	**45 Millions**
STEP 1	62 Millions short reads (**88%**)	60 Millions short reads (**85%**)	38 Millions short reads (**85%**)
STEP 2	**84,94 %** mapped in PE	**48,20 %** mapped in PE	**83,11 %** mapped in PE
SNPs	**847.130**	**3.245.011**	**532.756**

0/ I start with short reads in paired-ends (101 nucleotides).

1/ I run one workflow to filter and trim all my short reads in input files.

⇨ 15 % of short reads are removed for **ALL** species.

2/ I run MAPHiTS.

⇨ 85 % of short reads are mapped in paired-ends for **A** and **C** but only 48% for **B**.

I've got 500.000 SNPs for **A** and **C** and 3 millions for **B** !

➡ **A** and **C** are closest to reference genome than **B**.

VI. Perspectives

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- **Add new tools** (all tools used in all our pipelines)
- **Link Galaxy to a visualization software** (Gbrowse 2, Tablet, GenomeView, …)
- **Application Note in progress (2011)**

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- Galaxy community

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