

# Development of a workflow for SNPs detection in grapevine species: MAPHiTS

-> *Integration of MAPHiTS in Galaxy*



## A. Galaxy Presentation



# A.1. Galaxy Homepage



The screenshot shows the Galaxy web interface. The top navigation bar includes 'Galaxy' (with a logo), 'Tools' (dropdown), 'Options', 'Analyze Data', 'Workflow', 'Shared Data', 'Help', and 'User'. The left sidebar lists various tools: 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, and Workflows. The main content area features the URGI logo (green stylized leaves and the word 'URGI') surrounded by DNA helixes. A box labeled 'Unité Recherche Génomique Info' is present. The right sidebar shows a history panel titled 'History' with 'Unnamed history' and a message: 'Your history is empty. Click 'Get Data' on the left pane to start'. The INRA logo (green globe and the word 'INRA') is also visible.

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

## A.2. 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 management) and a PostgreSQL Database (for Galaxy).

# A.3. Homepage presentation

Return to  
homepage

Tools

Management  
of Galaxy

History



The screenshot shows the URGI homepage. On the left is a sidebar titled "Tools" with various genomic analysis options. The main content area features the URGI logo and text for the "Unité Recherche Génomique Info" with a link to <http://urgi.versailles.inra.fr/>. The right side includes a "Management of Galaxy" section and a "History" panel.

Annotations:

- A red line with an arrow points from the "Return to homepage" text to the "Galaxy" button in the top navigation bar.
- A blue line with an arrow points from the "Tools" text to the "Tools" sidebar.
- An orange line with an arrow points from the "Management of Galaxy" text to the "Management of Galaxy" section in the main content area.
- A green line with an arrow points from the "History" text to the "History" panel on the right.

# A.4. How to upload your data ?

a. Tools → Get Data → Upload File

Galaxy

Tools

**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 Test server
- GrameneMart Central server
- Flymine server
- Flymine test server
- modMine server
- Ratmine server
- Wormbase server
- Wormbase test server
- EuPathDB server
- EncodeDB at NHGRI
- EpiGRAPH server
- EpiGRAPH test server
- HbVar Human Hemoglobin Variants and Thalassemias

Send Data

ENCODE Tools

Lift-Over

Text Manipulation

Filter and Sort

Join, Subtract and Group

Convert Formats

Extract Features

Fetch Sequences

Fetch Alignments

Get Genomic Scores

Operate on Genomic Intervals

Analyze Data Workflow Data Libraries Help User

Upload File

File Format:

Auto-detect

Which format? See help below

File:

Parcourir...

URL/Text:

Here you may specify a list of URLs (one per line) or paste the contents of a file.

Convert spaces to tabs:

Yes

Use this option if you are entering intervals by hand.

Genome:

Click to Search or Select Build

Execute

Help

Auto-detect

The system will attempt to detect Axt, Fasta, Fastqsolexa, Gff, Gff3, Html, Lav, Maf, Tabular, Wiggle, Bed and Interval (Bed with headers) formats. If your file is not detected properly as one of the known formats, it most likely means that it has some format problems (e.g., different number of columns on different rows). You can still coerce the system to set your data to the format you think it should be. You can also upload compressed files, which will automatically be decompressed.

Ab1

A binary sequence file in 'ab1' format with a '.ab1' file extension. You must manually select this 'File Format' when uploading the file.

Axt

blastz pairwise alignment format. Each alignment block in an axt file contains three lines: a summary line and 2 sequence lines. Blocks are separated from one another by blank lines. The summary line contains chromosomal position and size information about the alignment. It consists of 9 required fields.

Bam

A binary file compressed in the BGZF format with a '.bam' file extension.

History

Unnamed history

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

## A.4. How to upload your data ?

The screenshot shows the Galaxy web interface with a red box highlighting the message bar and the history panel.

**Message Bar:**

- Your upload has been queued. History entries that are still uploading will be blue, and turn green upon completion.
- Please do not use your browser's "stop" or "reload" buttons until the upload is complete, or it may be interrupted.
- You may safely continue to use Galaxy while the upload is in progress. Using "stop" and "reload" on pages other than Galaxy is also safe.

**History Panel:**

- Unnamed history
- 2: Genome\_Tomate.fasta

**Tool Panel:**

- Get Data
- Send Data
- ENCODE Tools
- Lift-Over
- Text Manipulation
- Filter and Sort
- Join, Subtract and Group
- Convert Formats
- Extract Features
- Fetch Sequences
- Fetch Alignments
- Get Genomic Scores
- Operate on Genomic Intervals
- Statistics
- Graph/Display Data
- Regional Variation
- Multiple regression
- Multivariate Analysis
- Evolution
- Metagenomic analyses
- FASTA manipulation
- NGS: QC and manipulation
- NGS: Mapping
- NGS: SAM Tools
- NGS: Peak Calling
- Rg Data
- Rg Simulate
- Rg Visualise
- Rg Model Data
- MyTools
- Workflows

**Workflow Status:**

- Waiting:** 5: (BWA) Output SAM
- Ongoing:** 2: Genome\_Tomate.fasta
- Finished → Error:** 5: (BWA) Output SAM
- Finished → OK:** 3: Genome\_Tomate.fasta

# A.5. How to use a tool ?

## a. Choose a tool from the list

The screenshot shows the Galaxy web interface with the following components:

- Header:** Galaxy, Analyze Data, Workflow, Data Libraries, Help, User.
- Left Sidebar (Tools):**
  - Get Data
  - Send Data
  - ENCODE Tools
  - Lift-Over
  - Text Manipulation
  - Filter and Sort
  - Join, Subtract and Group
  - Convert Formats
  - Extract Features
  - Fetch Sequences
  - Fetch Alignments
  - Get Genomic Scores
  - Operate on Genomic Intervals
  - Statistics
  - Graph/Display Data
  - Regional Variation
  - Multiple regression
  - Multivariate Analysis
  - Evolution
  - Metagenomic analyses
  - FASTA manipulation
  - NGS: QC and manipulation
  - NGS: Mapping** (highlighted)
    - Map with Bowtie for Illumina (highlighted)
    - Megablast compare short reads against htgs, ht, and wgs databases
    - Parse blast XML output  - NGS: SAM Tools
  - NGS: Peak Calling
  - Rg Data
  - Rg Simulate
  - Rg Visualise
  - Rg Model Data
  - MyTools
  - Workflows
- Tool Configuration Page:**

Map with Bowtie for Illumina

Will you select a reference genome from your history or use a built-in index?: Use a built-in index (selected)

Select a reference genome: (dropdown menu)

Is this library mate-paired?: Single-end (selected)

FASTQ file: (dropdown menu)

Bowtie settings to use: Commonly used (selected)

Suppress the header in the output SAM file: (checkbox checked)

**Execute** button (highlighted with a red arrow)

**What it does:** Bowtie is a short read aligner designed to be ultrafast and memory-efficient. It is developed by Ben Langmead and Cole Trapnell. Please cite: Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology* 10:R25.

**Know what you are doing:** There is no such thing (yet) as an automated gearshift in short read mapping. It is all like stick-shift driving in San Francisco. In other words = running this tool with default parameters will probably not give you meaningful results. A way to deal with this is to understand the parameters by carefully reading the documentation and experimenting. Fortunately, Galaxy makes experimenting easy.

**Input formats:** Bowtie accepts files in Sanger FASTQ format. Use the FASTQ Groomer to prepare your files.

**Outputs:** The output is in SAM format, and has the following columns:

Column	Description
1 NAME	Query (pair) NAME
2 FLAG	hitwise FLAG
- Right Sidebar (History):**
  - Unnamed history
  - Your history is empty. Click 'Get Data' on the left pane to start

b. Set options and parameters

c. Execute Help

# A.6. Example

Galaxy

Analyze Data Workflow Shared Data Admin Help User

Tools Options ▾

- Lift-Over
- Text Manipulation
- Filter and Sort
- 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: Mapping
- NGS: Indel Analysis
- NGS: Expression Analysis
- NGS: SAM Tools
- NGS: Peak Calling
- SNP/WGA: Data; Filters
- SNP/WGA: QC; LD; Plots
- SNP/WGA: Statistical Models
- Human Genome Variation
- URGI Mapping BES
- URGI Others Tools
- MAPHITS
- SMART
- Get Letter Distribution Calculate distribution for each nucleotide per position for all short reads (S-MART)

Workflows

History Options ▾

Smart

27: Get Letter Distribution on data 21

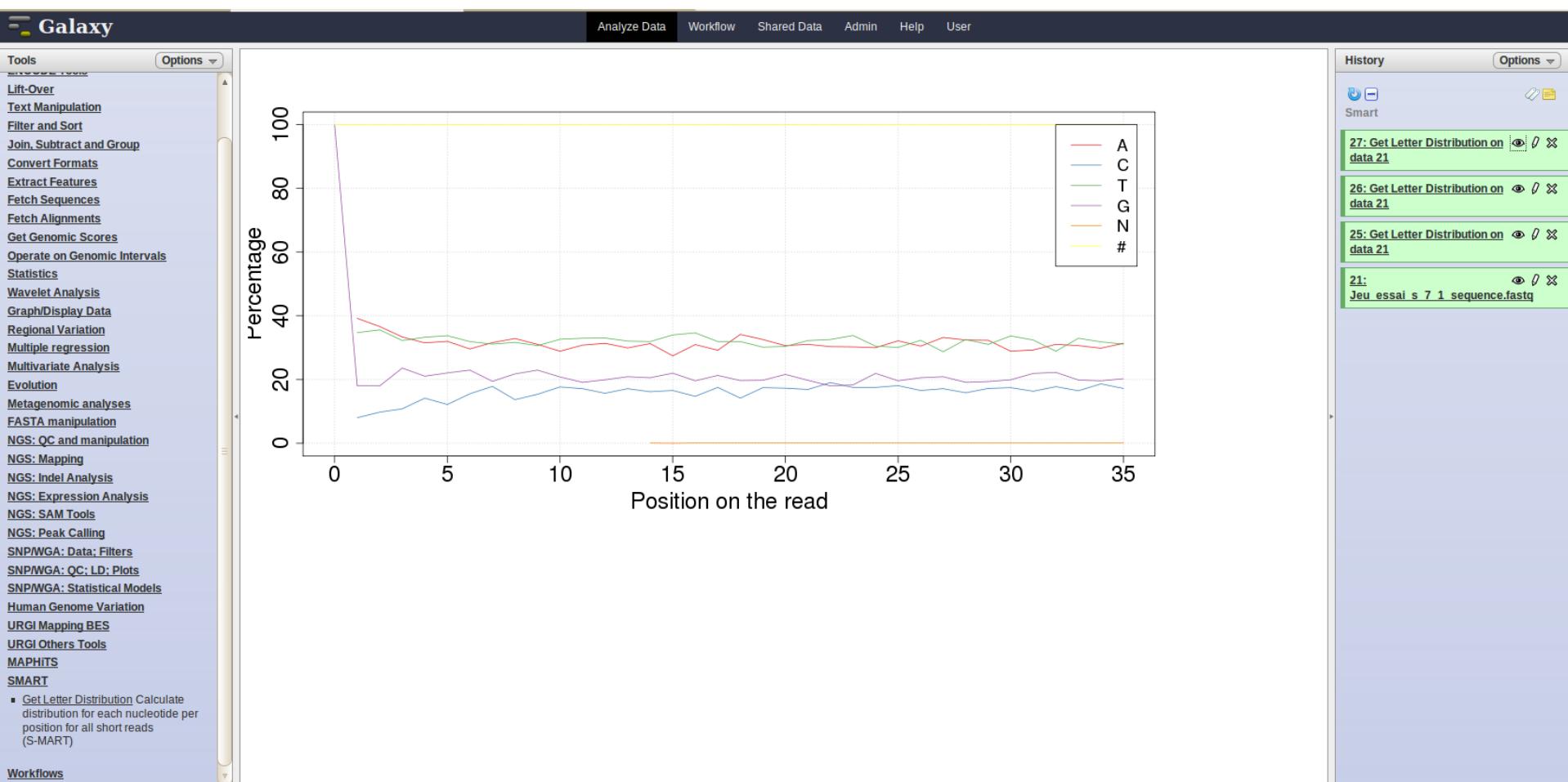
26: Get Letter Distribution on data 21

25: Get Letter Distribution on data 21

21: Jeu essai s 7 1 sequence fastq

## SMART - Get Letter Distribution

# A.6. Example



## SMART - Get Letter Distribution

## B. MAPHiTS Presentation



## B.1. 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:**

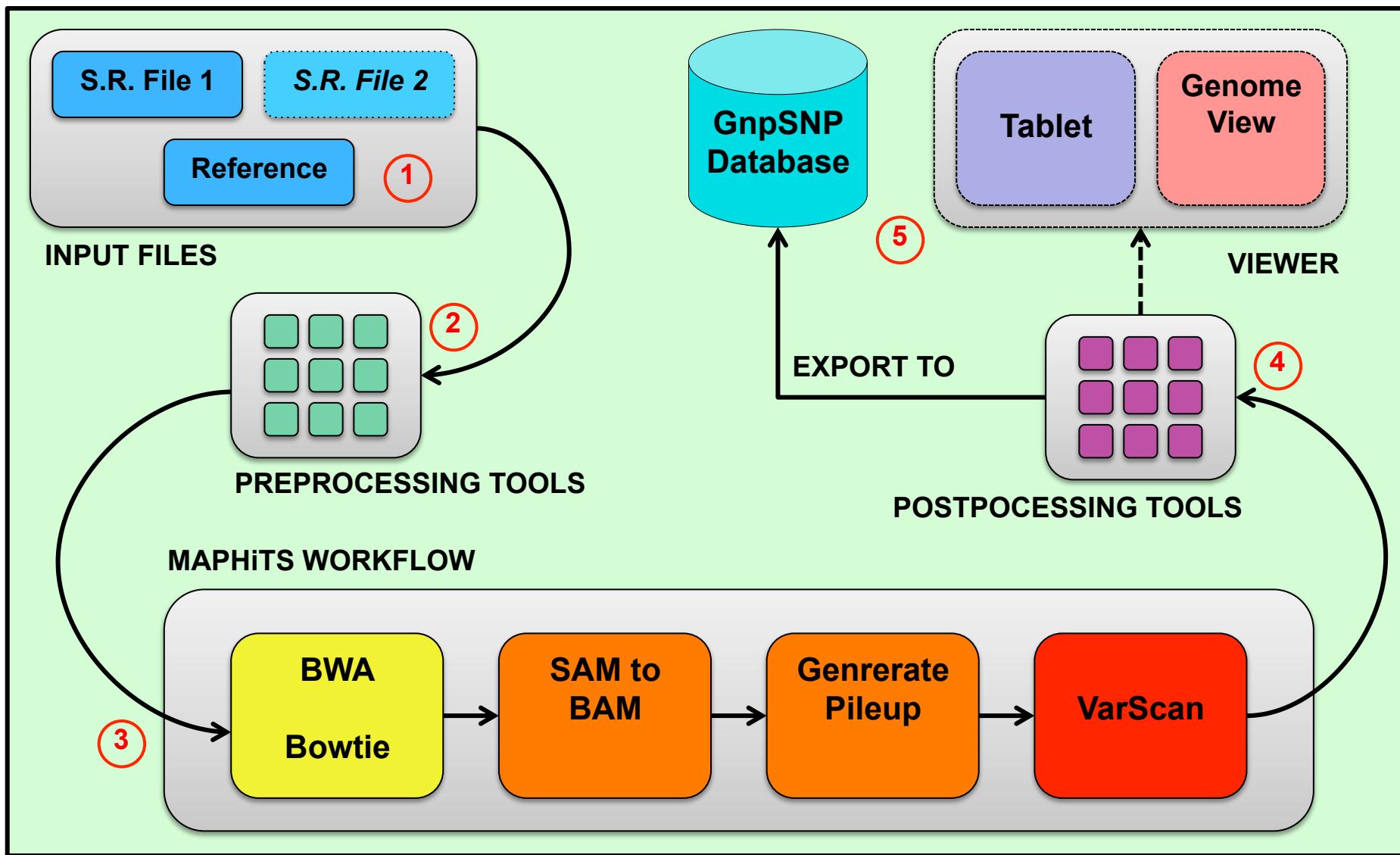
- Muscaries : 6 genotypes
  - GrapeReSeq : 16 genotypes

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



Other projects are also in progress with others species.

## B.2. MAPHiTS: Resume



## B.3. 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

Fewer Ressources Required !

Exemple:

- Before: 1 big job in 1 cluster node
- Now: N little jobs in N cluster nodes

## B.3. MAPHiTS Development Tools

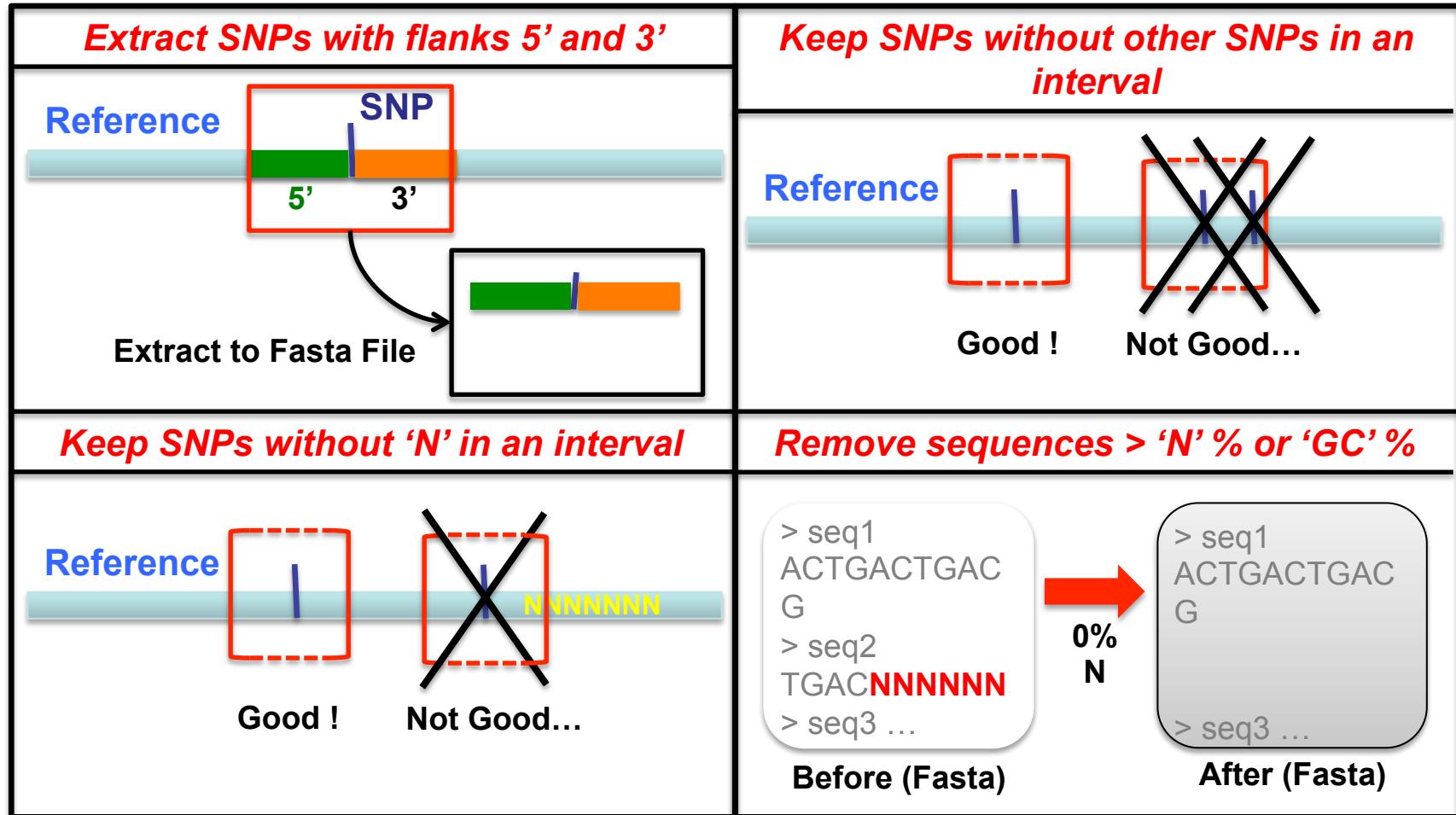
### ■ Preprocessing tools:

- Remove duplicated short-reads
- Remove short reads not in paired-ends
- Remove short reads > 'N'%
- Remove informations in each FASTA file header

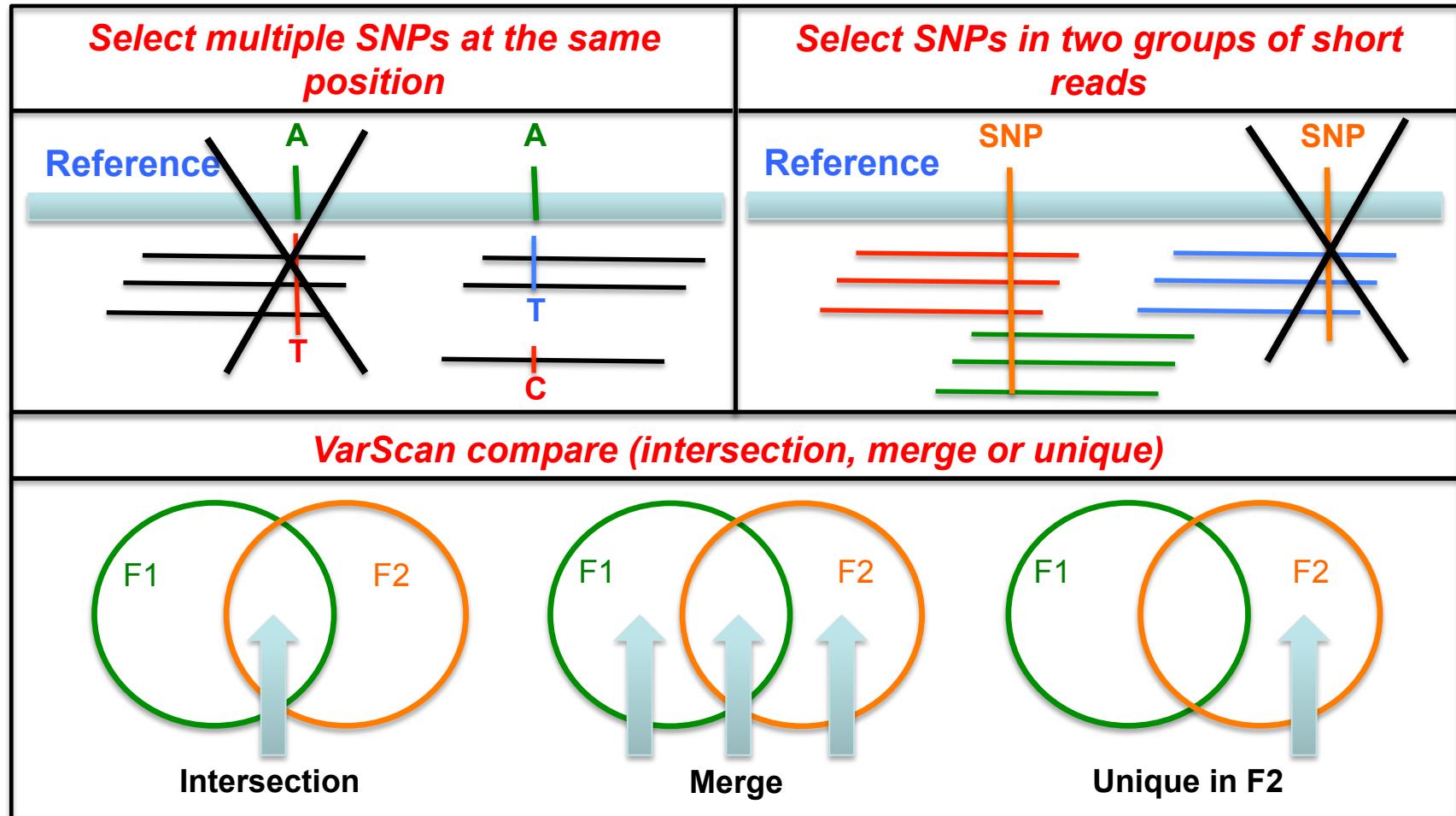
### ■ Postprocessing tools:

- Count multiple hits from the results of BWA
- Extract short reads from SAM file
- VarScan compare (multiple files)
- VarScan filter
- VarScan to Gff3

## B.3. MAPHiTS Development Tools



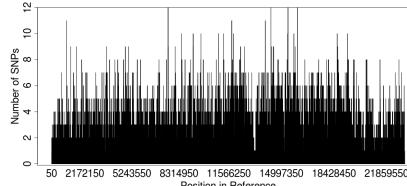
## B.3. MAPHiTS Development Tools



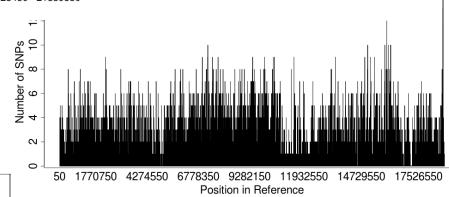
## B.3. MAPHiTS Development Tools

**Get SNPs distribution by chromosome**

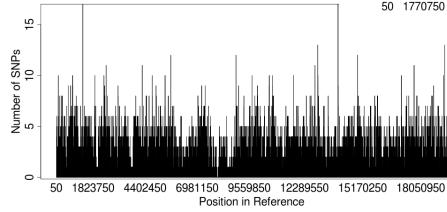
VarScan File



Graph chr1



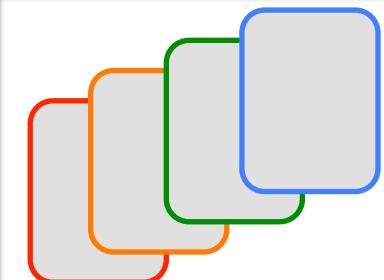
Graph chr2



Graph chr3

**Give consensus nucleotides (with different VarScan analysis)**

VarScan Files



HTML File

INRA - URGi Team

MAPHiTS Genotype Card

Ref	Position	RefAllele	Trayshed	Vitis-Rotundifolia	Regale_run2	Regale_run1	Fry	Carlos	Consensus
chr18	801	G	T	T	G	G	T	T	NA
chr18	807	C	T	T	C	C	T	T	NA
chr18	977	A	A	A	A	A	A	T	NA
chr18	1060	T	T	T	T	T	T	A	NA
chr18	1751	A	A	A	A	A	A	G	NA
chr18	1752	A	A	A	A	A	A	T	NA
chr18	1786	A	A	A	A	A	A	G	NA
chr18	1890	T	T	T	T	T	T	G	NA
chr18	2586	T	T	T	T	T	T	G	NA
chr18	2591	T	G	G	T	T	T	G	NA
chr18	4499	A	G	G	G	G	G	G	G 6%
chr18	4534	T	A	A	A	T	A	A	NA
chr18	4585	G	C	G	C	G	C	C	NA
chr18	4612	C	T	C	T	T	T	C	NA
chr18	4618	T	C	T	C	C	C	C	NA
chr18	4665	A	G	G	G	G	G	G	G 6%
chr18	4754	T	C	C	C	C	C	C	C 6%
chr18	4785	C	A	C	A	A	A	A	NA
chr18	4813	A	G	G	G	G	G	G	G 6%
chr18	4824	A	T	T	T	T	T	T	T 6%
chr18	4910	A	G	G	G	A	A	G	NA

## B.3. MAPHiTS Development Tools

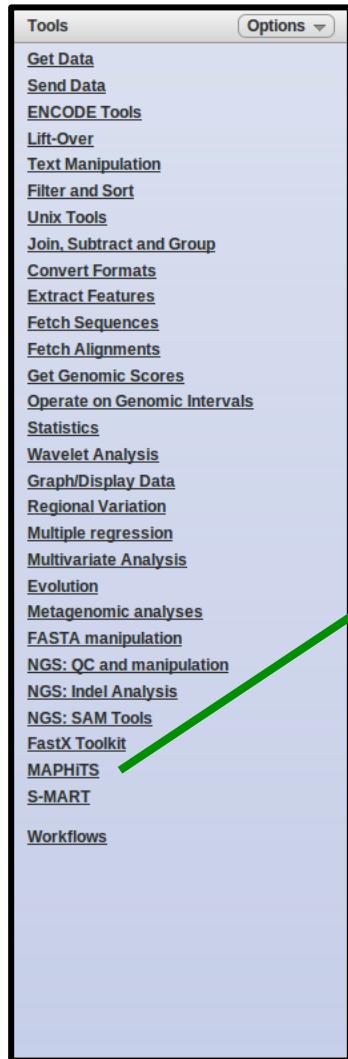
MAPHiTS Genotype Card

Ref	Position	RefAllele	Trayshed	Vitis-Rotundifolia	Regale_run2	Regale_run1	Fry	Carlos	Consensus
chr18	801	G	T	T	G	G	T	T	NA
chr18	807	C	T	T	C	C	T	T	NA
chr18	977	A	A	A	A	A	A	T	NA
chr18	1060	T	T	T	T	T	T	A	NA
chr18	1751	A	A	A	A	A	A	G	NA
chr18	1752	A	A	A	A	A	A	T	NA
chr18	1786	A	A	A	A	A	A	G	NA
chr18	1890	T	T	T	T	T	T	G	NA
chr18	2586	T	T	T	T	T	T	G	NA
chr18	2591	T	G	G	T	T	T	G	NA
chr18	4499	A	G	G	G	G	G	G	G 6/6
chr18	4534	T	A	A	A	T	A	A	NA
chr18	4585	G	C	G	C	G	C	C	NA
chr18	4612	C	T	C	T	T	T	C	NA
chr18	4618	T	C	T	C	C	C	C	NA
chr18	4665	A	G	G	G	G	G	G	G 6/6
chr18	4754	T	C	C	C	C	C	C	C 6/6
chr18	4785	C	A	C	A	A	A	A	NA
chr18	4813	A	G	G	G	G	G	G	G 6/6
chr18	4824	A	T	T	T	T	T	T	T 6/6
chr18	4910	A	G	G	G	A	A	G	NA

## C. MAPHiTS in Galaxy



## C.2. New URGI Integrated tools



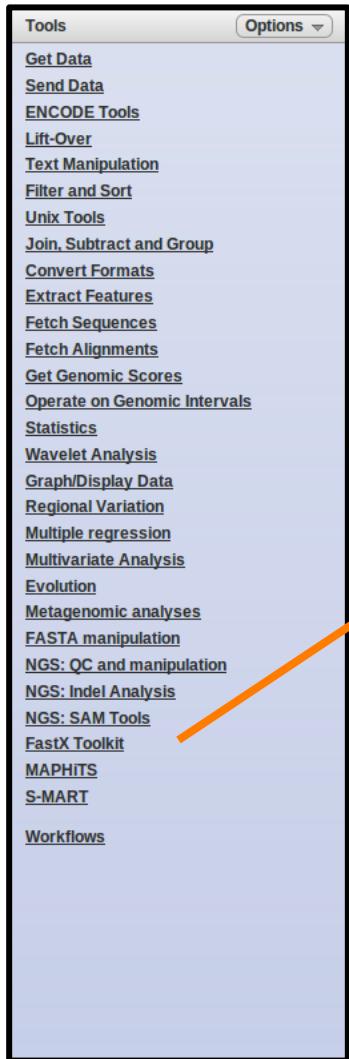
### MAPHiTS

#### 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)

## C.2. New Integrated tools



FASTX-Toolkit

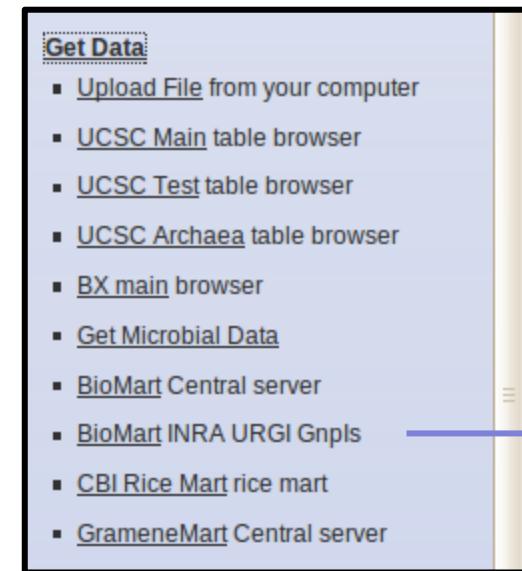
The screenshot shows the FastX Toolkit software interface. The title bar says 'FastX Toolkit'. Below it, the word 'TOOLS' is centered. A list of tools is provided, each preceded by a blue square bullet point:

- [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](#)

## C.2. New Others URGI

# Integrated tools

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



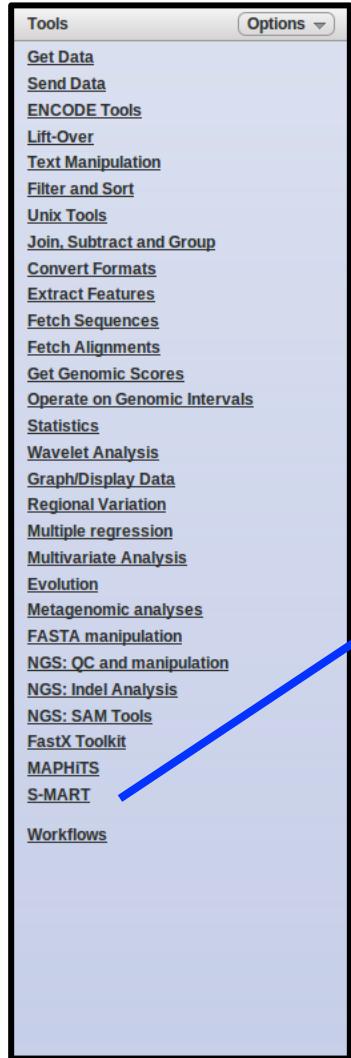
**BioMart**  
**URGI**  
**Gnpls**

The screenshot shows the GnpIS advanced search interface. It includes:

- URGI logo and text "GnpIS advanced search".
- A navigation bar with buttons for "New", "Count", and "Results".
- A "Dataset" selection field showing "[None selected]".
- A "CHOOSE DATABASE" dropdown menu.

## C.2. New Others URGI

# Integrated tools



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.

## C.2. New Others URGI Integrated tools

What can I do with all this RNA-Seq data ?

S-MART:

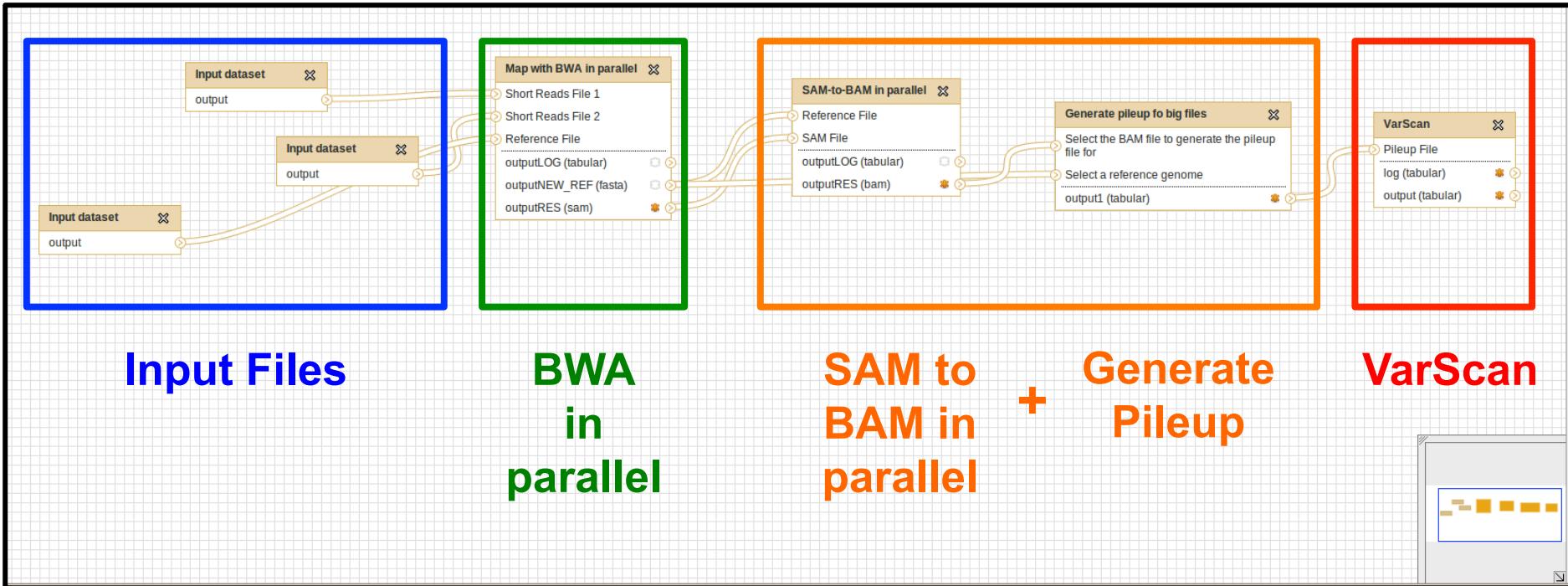
- is a set of independant tools
- works on a standard PC and with Galaxy
- can be installed and used easily

Use S-MART for data manipulation, data visualization, differential expression, ...

**Link:** <http://urgi.versailles.inra.fr/Tools/S-MART>

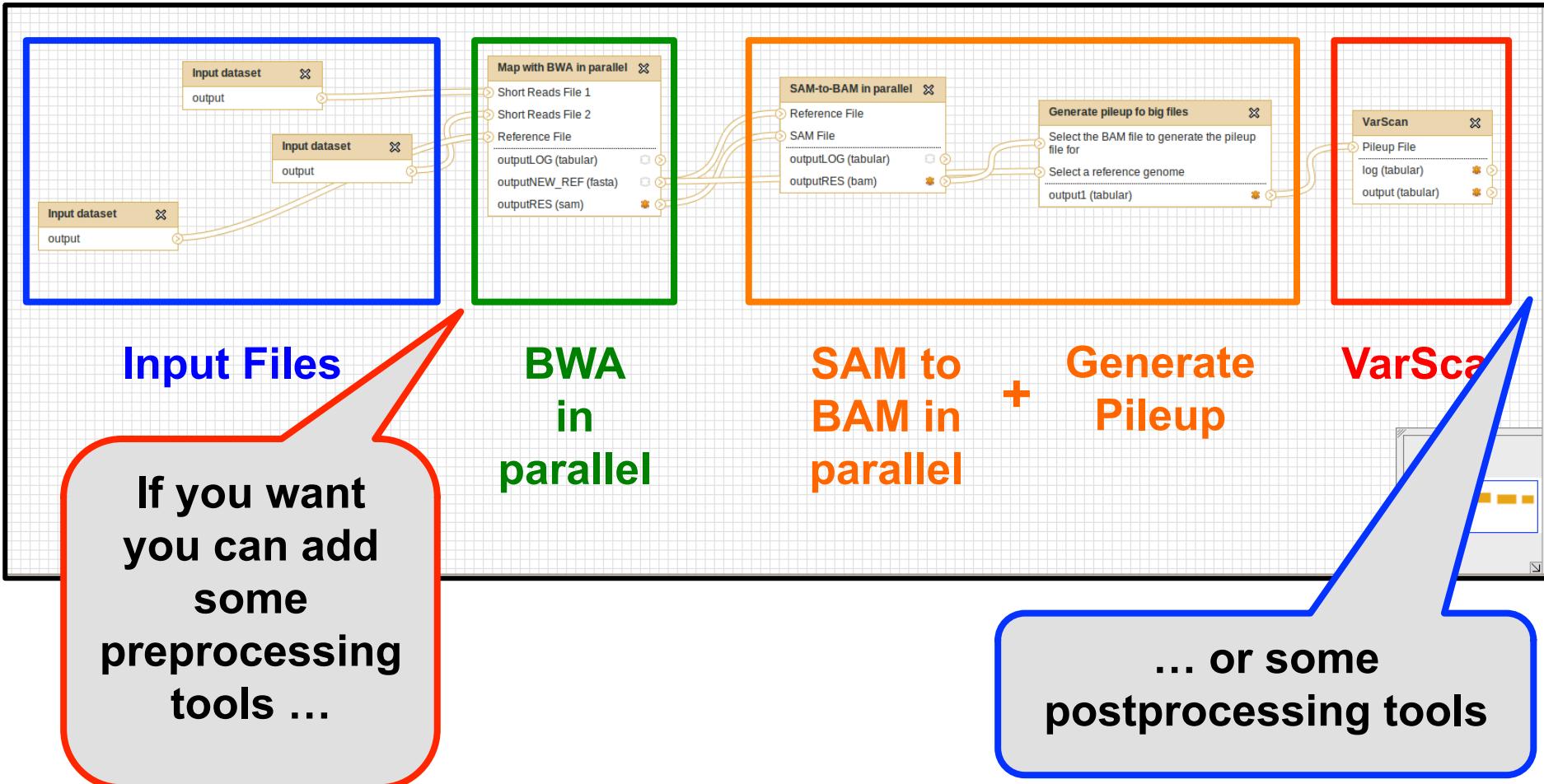
**Contact:** [matthias.zytnicki@versailles.inra.fr](mailto:matthias.zytnicki@versailles.inra.fr)

# C.3. MAPHiTS: Build

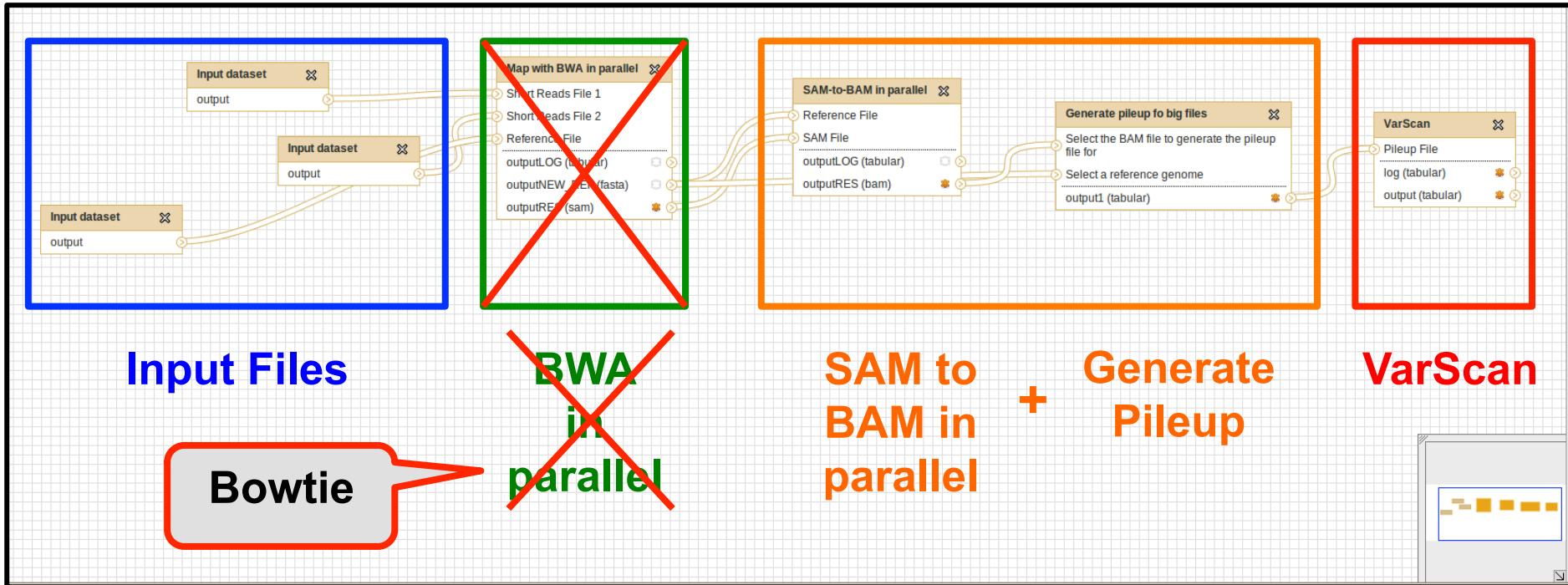


MAPHiTS is build using the graphical interface of Galaxy.

# C.3. MAPHiTS: Build



# C.3. MAPHiTS: Build



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

# C.4. MAPHiTS: Launch

Running workflow "MAPHiTS Parallel (paired)"

Step 1: Input dataset  
Reference File (.fasta)

Step 2: Input dataset  
Short Reads File 1 (.fastq)

Step 3: Input dataset  
Short Reads File 2 (.fastq)

Step 4: Map with BWA in parallel  
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)  
 ← 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

# C.4. MAPHiTS: Launch

Galaxy

Analyze Data Workflow Shared Data Help User

Tools

- [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

- [Trim And Compare ALL Short Reads \(paired\)](#)
- [MAPHiTS Not Parallel \(single\)](#)
- [MAPHiTS Not Parallel \(paired\)](#)
- [MAPHiTS Parallel \(single\)](#)
- [MAPHiTS Parallel \(paired\)](#)
- [Trim And Compare EPGV Short Reads \(paired\)](#)
- [All workflows](#)

History Options

Workshop 6

- 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

When you run the workflow, this message appears !

# C.4. MAPHiTS: Launch

Galaxy

Analyze Data Workflow Shared Data Help User

Tools Options

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

- Trim And Compare ALL Short Reads (paired)
- MAPHiTS Not Parallel (single)
- MAPHiTS Not Parallel (paired)
- MAPHiTS Parallel (single)
- MAPHiTS Parallel (paired)
- Trim And Compare EPGV Short Reads (paired)
- All workflows

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

**VarScan results**

**Generate Pileup**  
**SAM-to-BAM**

**BWA**

**PreProcessing tool**

**Input files**

History Options

Workshop 6

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

## C.5. Shared Workflows

### Published Workflows

search  | [Advanced Search](#)

Name	Annotation
<a href="#">MAPHiTS Parallel (paired)</a>	Workflow of SNPs detection, in parallel, for paired-end short reads.
<a href="#">Trim And Compare EPGV Short Reads (paired)</a>	
<a href="#">Trim And Compare ALL Short Reads (paired)</a>	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.

## C.6. Shared your History

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

The screenshot shows a web-based application interface titled 'Published Histories'. At the top, there is a search bar with the placeholder 'search' and a magnifying glass icon, followed by a link to 'Advanced Search'. Below the search area is a table with two columns: 'Name' and 'Annotation'. The 'Name' column contains two entries: 'VarScan compare Muscaraes' and 'VarScan compare Muscaraes v2'. The 'Annotation' column is empty for both entries.

Name	Annotation
<a href="#">VarScan compare Muscaraes</a>	
<a href="#">VarScan compare Muscaraes v2</a>	

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

## C.7. Shared 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

## C.7. Shared Data

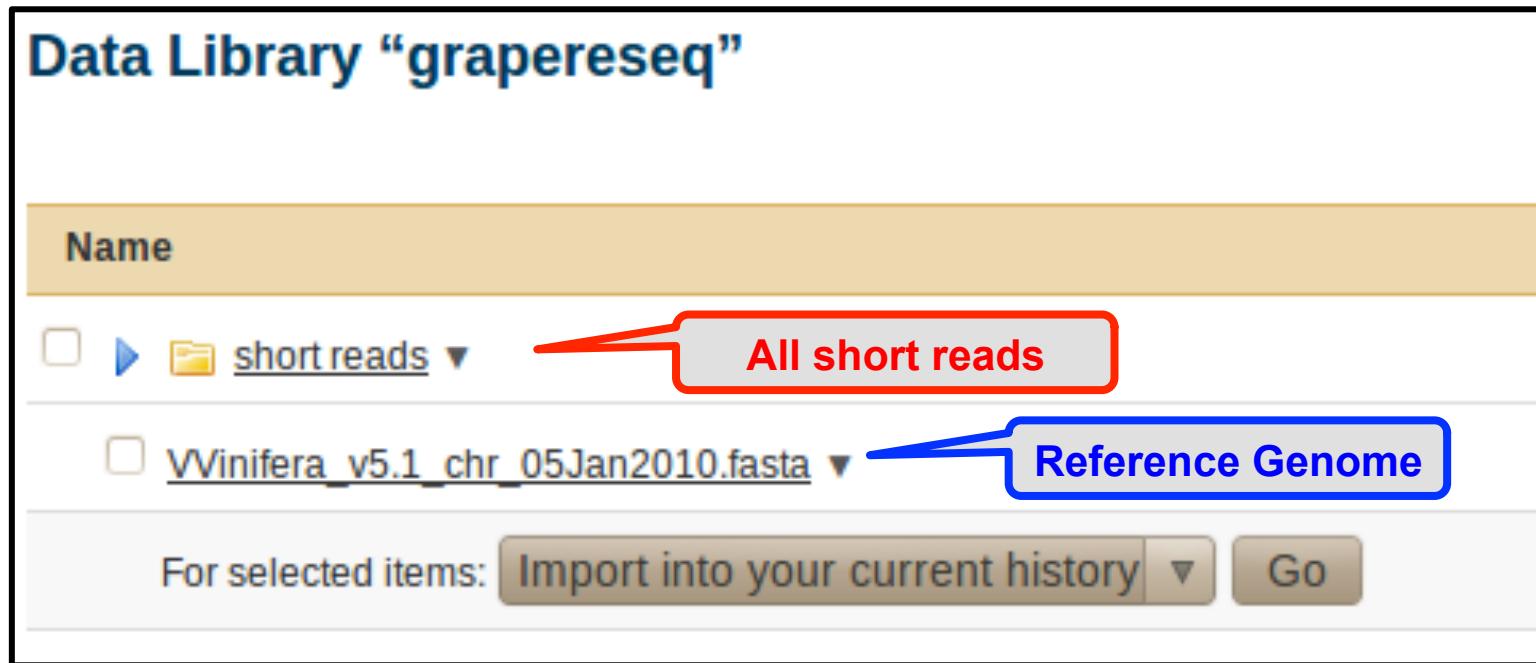
Data Library “grapereseq”

Name

➤  short reads ▾ All short reads

VVinifera\_v5.1\_chr\_05Jan2010.fasta ▾ Reference Genome

For selected items:  ▾



They can import their data into the history quickly.

→ Useful for NGS !

## D. Perspectives



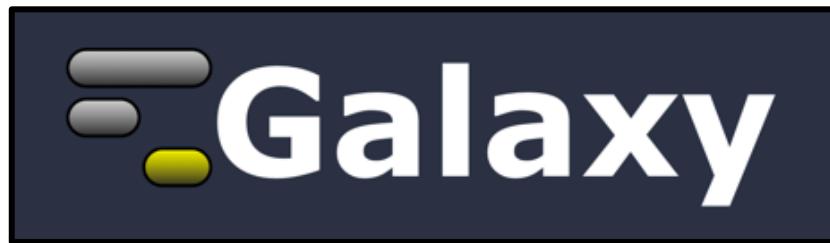
## D. Perspectives

- **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)**

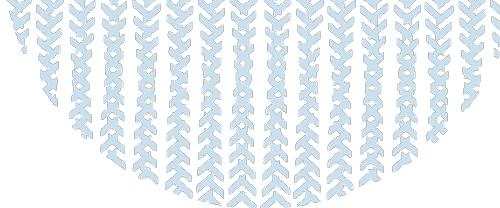
# Acknowledgements



- EPGV Team
- URGI Team



- Galaxy developers
- Galaxy community



# Thank you for your attention !!!

