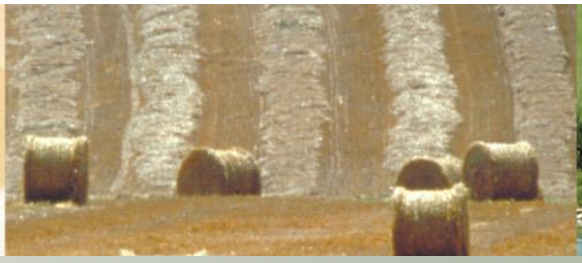
Development of a pipeline for SNPs detection

Colloque EPGV 2010:

Détection, Gestion et Analyse du Polymorphisme Des Génomes Végétaux






9, 10 et 11 Juin

ARNOUX Sandie
BRAS Marc

I. Background and objectives of the pipeline

Setting up a pipeline of SNPs detection to meet the needs of various projects.

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II. Rated software

• **BWA:** software of Mapping, particularly suitable for alignment of Short Reads against one sequence of reference (Burrows - Wheeler Alignment tool).
→ open source !!!

• **SAM tools:** toolkit for working on the output file of BWA.

• **VarScan:** software used to filter SNPs and indels from a BAM file, obtained from SAM tools.
→ predict SNPs / Indels / Seq. Cns.

• **Tablet:** software used to view differents file formats (GFF3, ACE, AFG, MAQ, SOAP, SAM et BAM).

• **GenomeView:** software used to view differents file formats (BAM, GFF, FASTA et annotation file).

II. Software selected
Format : Pileup vs VarScan

Pileup

C10HBa0111D09_LR276	99	T	24	,,,,.,,.,,,,,,,.,,,,,,^],^],	ZZZZTZZZXZZZZZZZZZZVXVUV
C10HBa0111D09_LR276	100	G	24	,$,,,.,,.,,,,,,,.,,,,,,,,	ZZZZZZZZZZZZZZZZZTTZVVVX
C10HBa0111D09_LR276	101	C	24	,,,.,$,.,,,,,,,.,,,,,,,^].	ZZZZZZZZZZZYLXZSQWZTQSQZ
C10HBa0111D09_LR276	102	C	23	,,,.,.,,,,,,.,,,,,,,.	ZZZZZZZYZZRZWZWJJROQUKZ
C10HBa0111D09_LR276	103	T	24	,$,,.,,,,,,,.,,,,,,,.^],	ZZZQZZZZZZZZZZZZZVVVVZU
C10HBa0111D09_LR276	104	T	27	,,.,,,,,,,.,,,,,,,.,^],^],^],^],	ZZTZZZZZZZZZZZZZZZVTZXVUVV
C10HBa0111D09_LR276	105	A	28	,$,$.,.,,,,,,,.,,,,,,,.,,,,,^F,	ZZKZTZZZZZZZZZZZZZZZXVUNXU
C10HBa0111D09_LR276	106	A	28	T,.,,,,,,,.,,,,,,,.,,,,,,^],^],	AZZZZZZZZZZZZZZZZZZXVZXXVTT

Reference Seq. Name

Position In Ref.

Base In this Position

Number Of Short Reads In this Position

Informations For All Short Reads

Base Quality

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II. Software selected
Format : Pileup vs VarScan

VarScan

Chrom	Position	Ref	Var	Reads1	Reads2	VarFreq	Strands1	Strands2	Qual1	Qual2	Pvalue
scaffold_1	7936	C	T	4	3	42,86%	2	1	28	35	0.1923076923076922
scaffold_1	14721	T	G	1	5	83,33%	1	2	39	30	0.015151515151515102
scaffold_1	28988	G	A	6	2	25%	2	2	30	38	0.4666666666666698
scaffold_1	59708	A	G	13	2	13,33%	2	1	29	22	0.48275862068965925
scaffold_1	60144	T	C	22	2	8,33%	2	1	35	34	0.48936170212766006
scaffold_1	60150	A	C	20	4	16,67%	2	2	30	31	0.10921985815602651
scaffold_1	60187	A	C	13	2	13,33%	2	1	34	36	0.48275862068965925
scaffold_1	60210	T	C	12	2	14,29%	2	1	34	26	0.48148148148148034

Min coverage: 8
Min reads2: 2
Min var freq: 0.01
Min avg qual: 15
P-value thresh: 0.99
Reading input from STDIN
332191236 bases in pileup file
7698459 met minimum coverage of 8x
86479 SNPs predicted

Different Filters

→ Choose your values !

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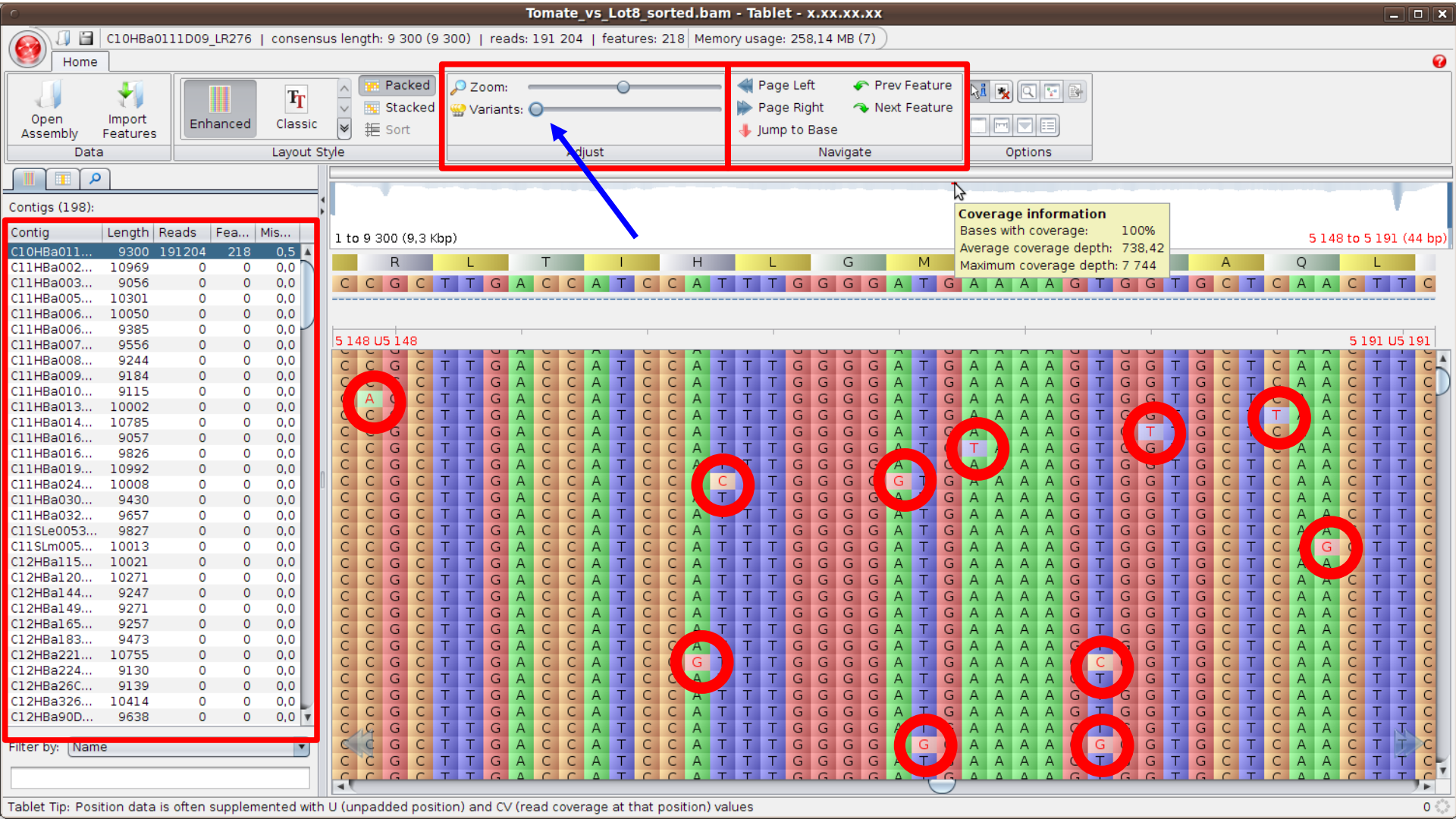
II. Software selected
Tablet: SNPs view



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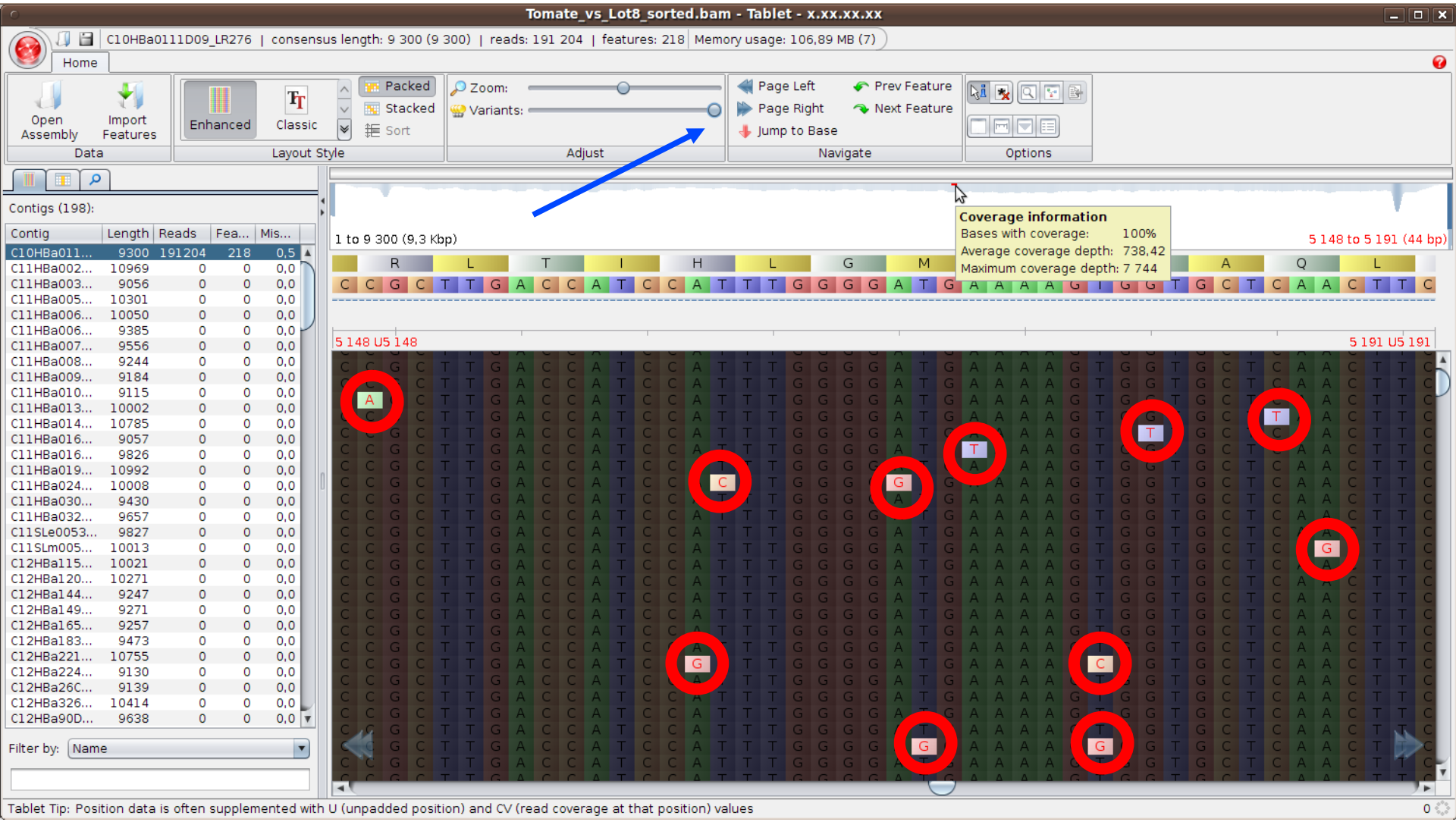
II. Software selected
Tablet: SNPs view



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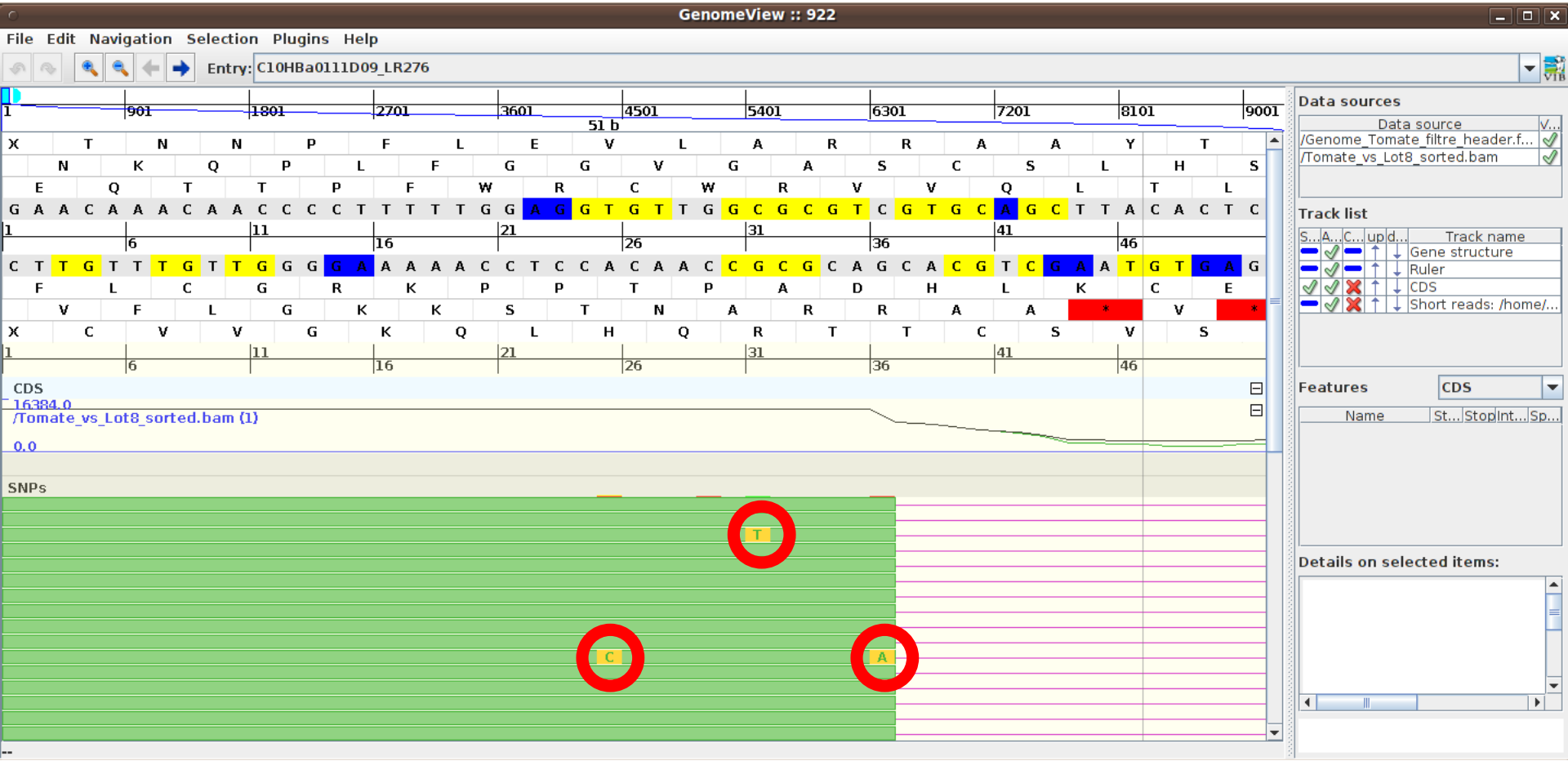
II. Software selected
GenomeView: SNPs view



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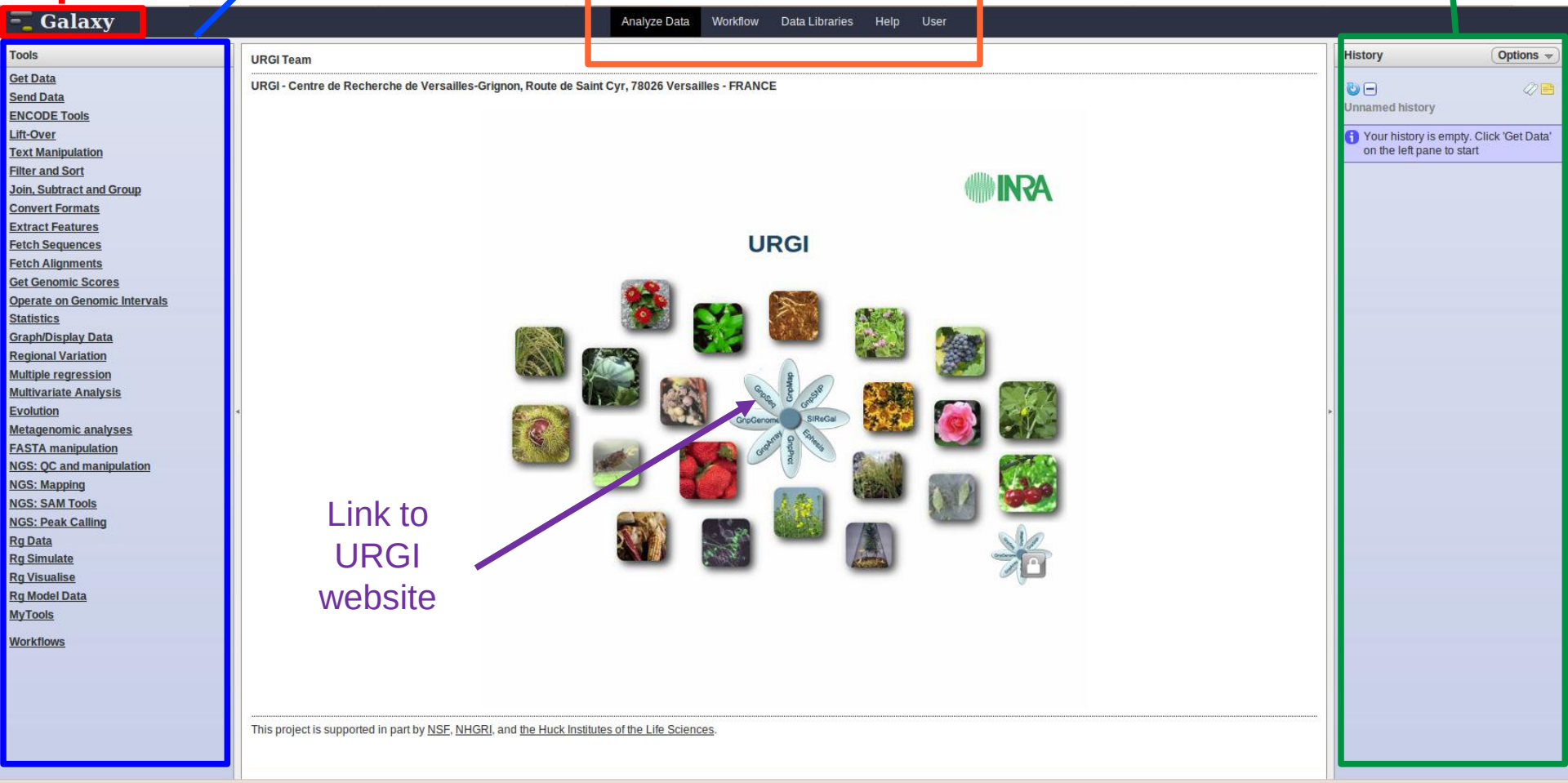
III. Integration of tools in Galaxy

Return to homepage

Tools

Managment of Galaxy

History



Link to URGI website

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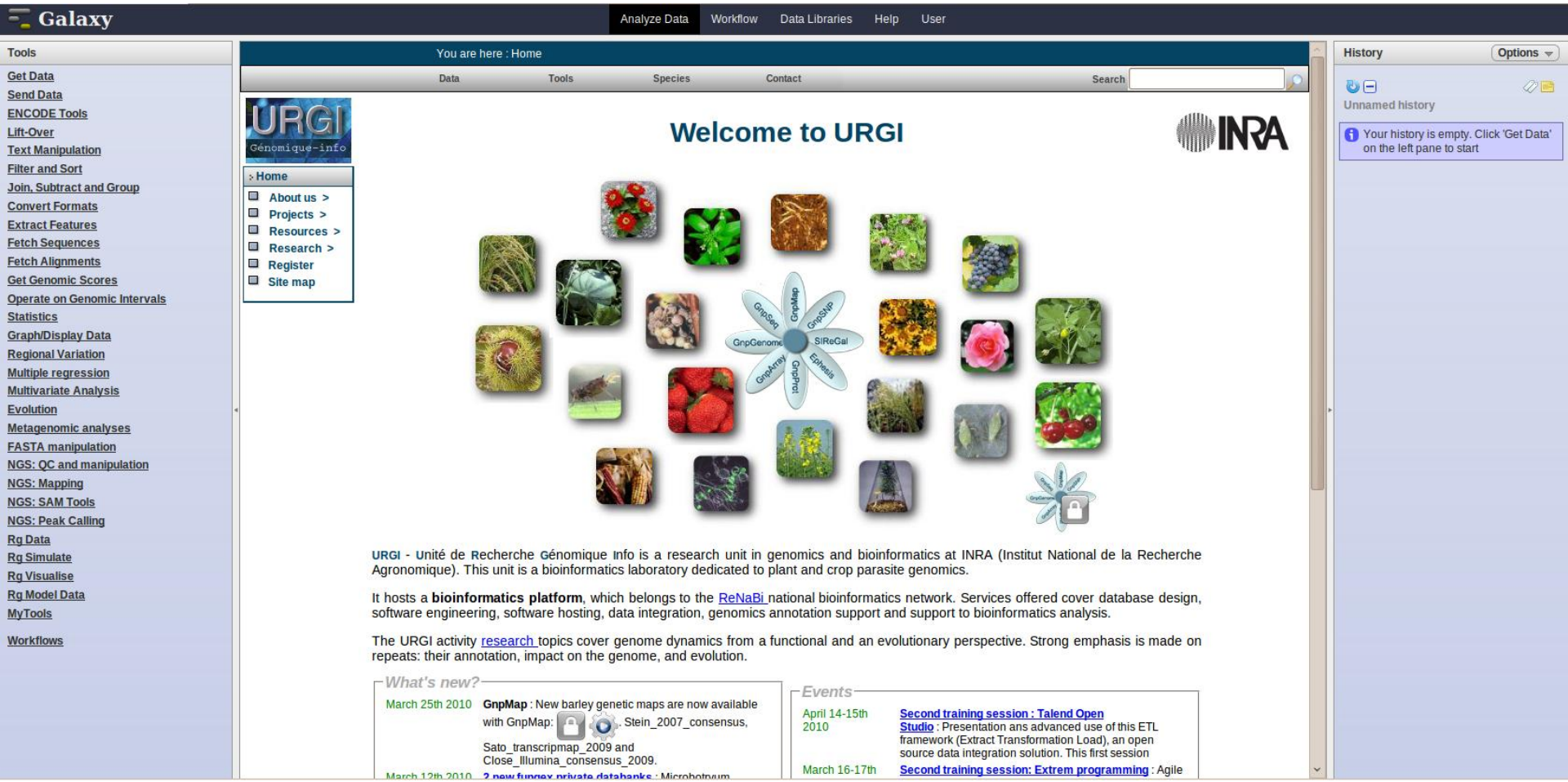
URGI public site

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



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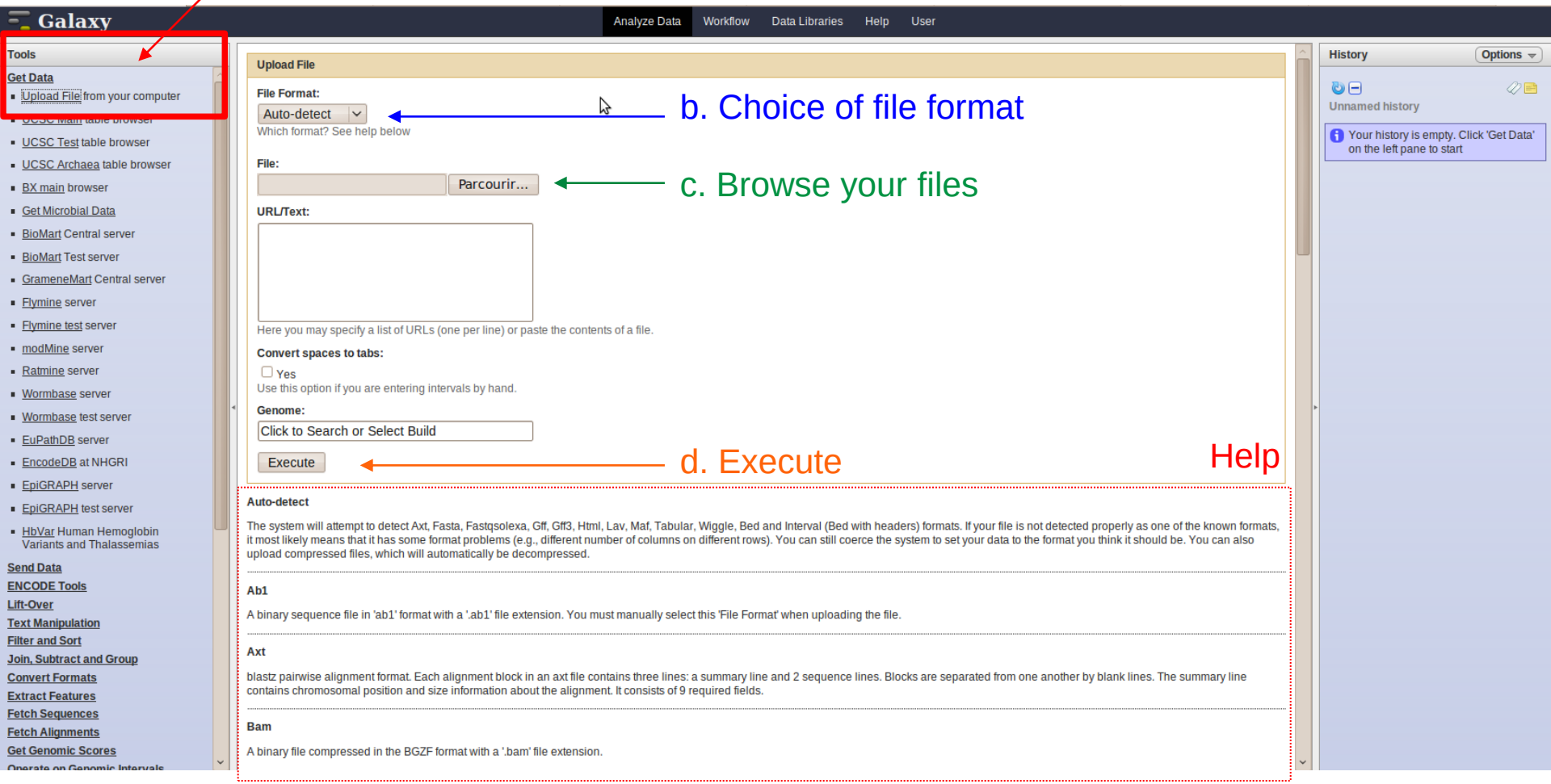
III.1. How to provide your data to Galaxy ?

a. Tools → Get Data → Upload File



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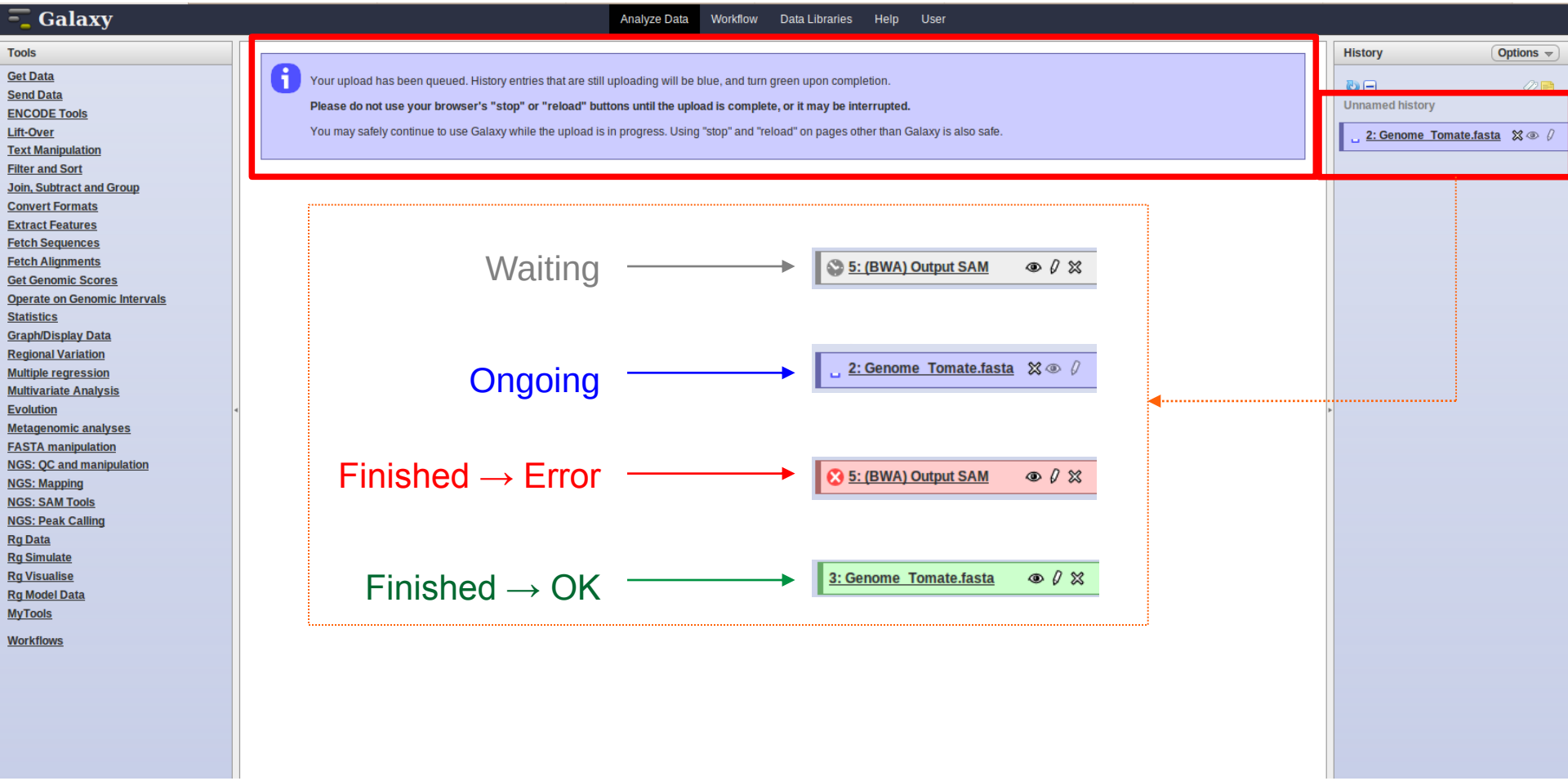

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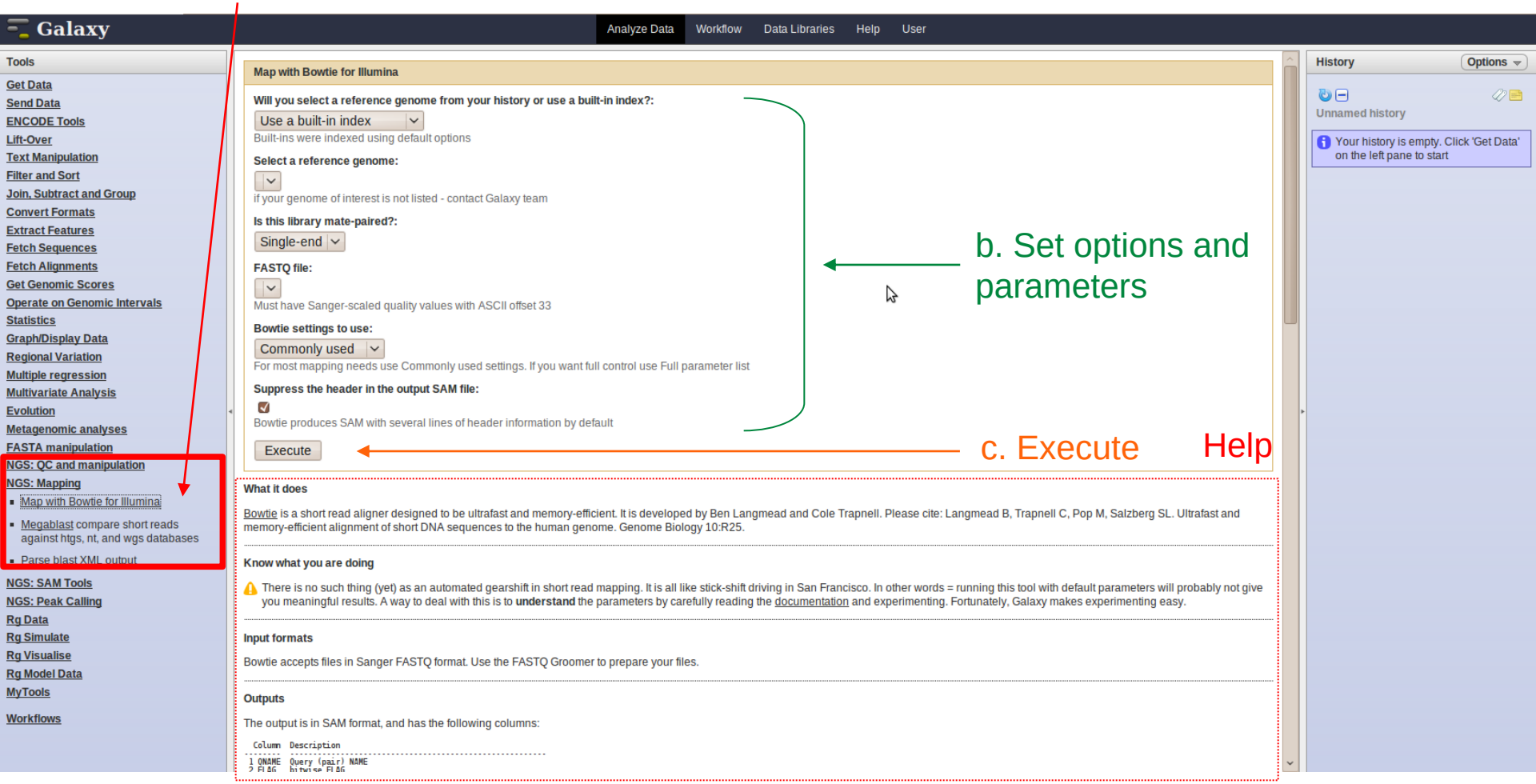
III.2. How to use a tool on Galaxy ?

a. Choose a tool from the list



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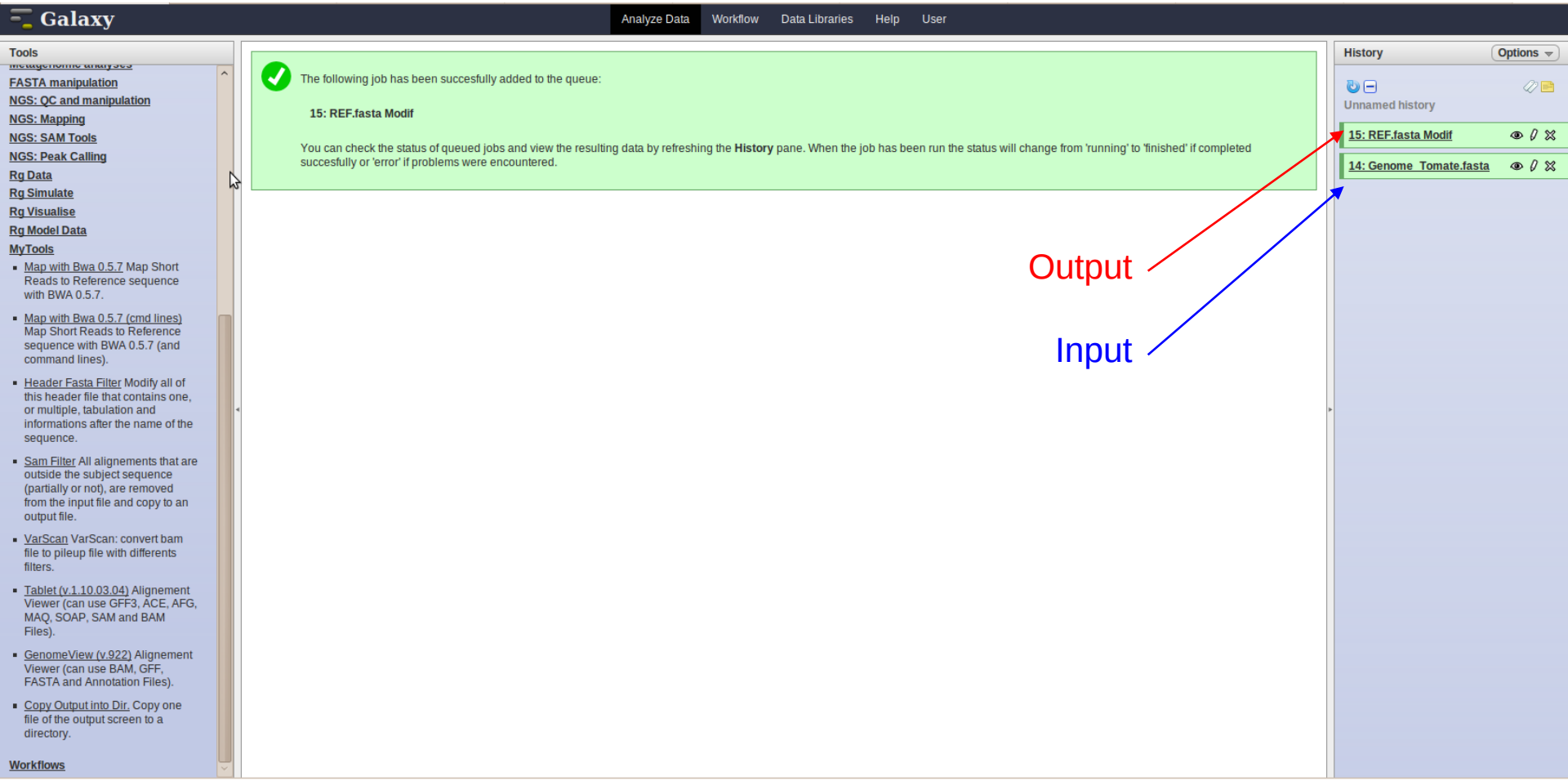

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III.3. How to integrate a tool in Galaxy ?

We can easily integrate a new tool !!!

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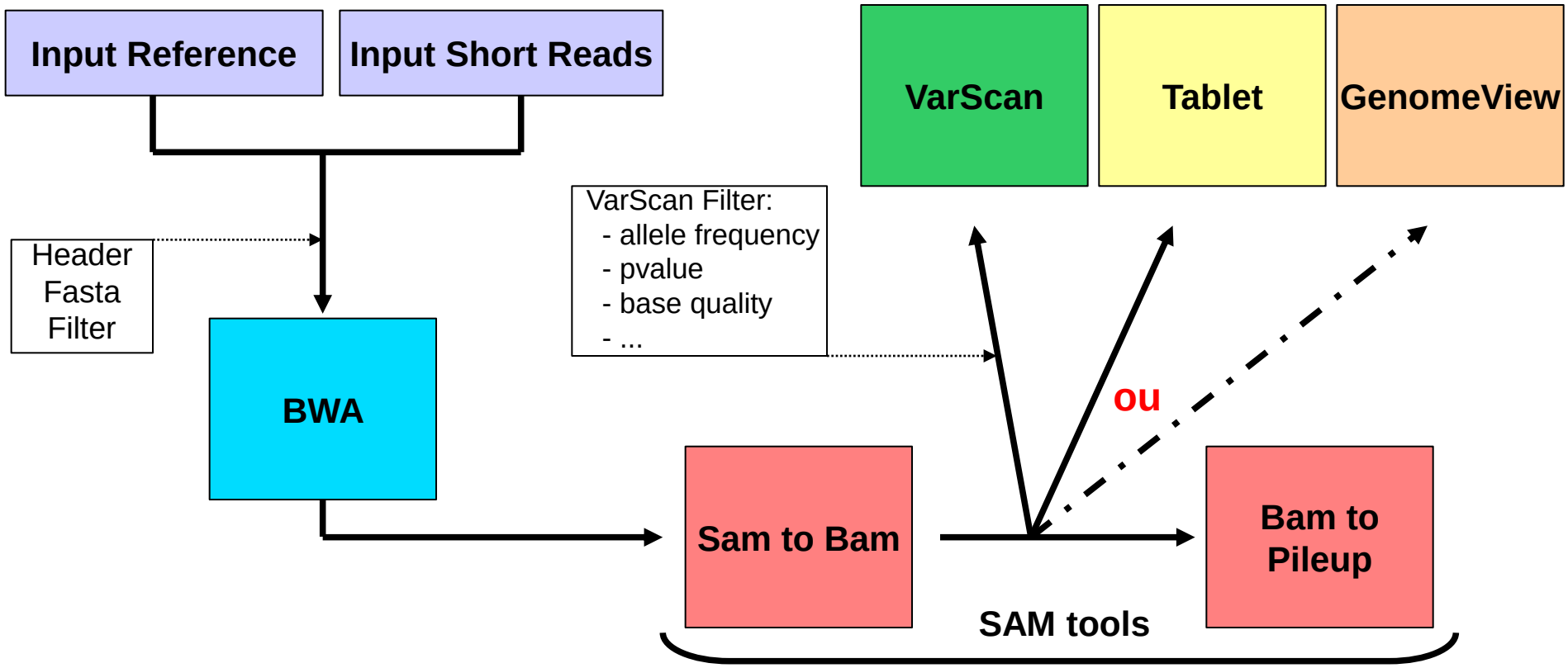
III.4. Our worklow for SNPs detection.



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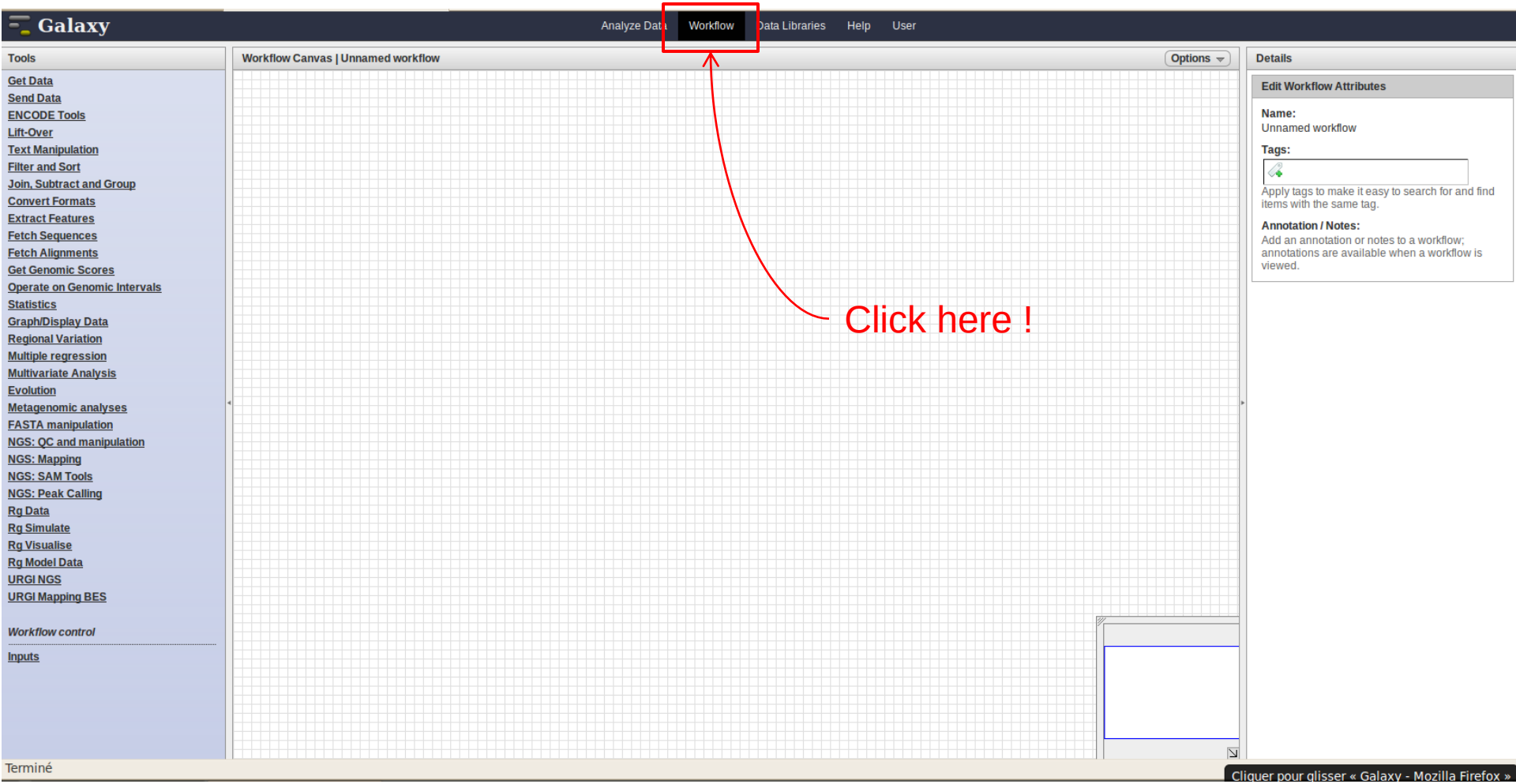
III.5. How to do this workflow with Galaxy ?



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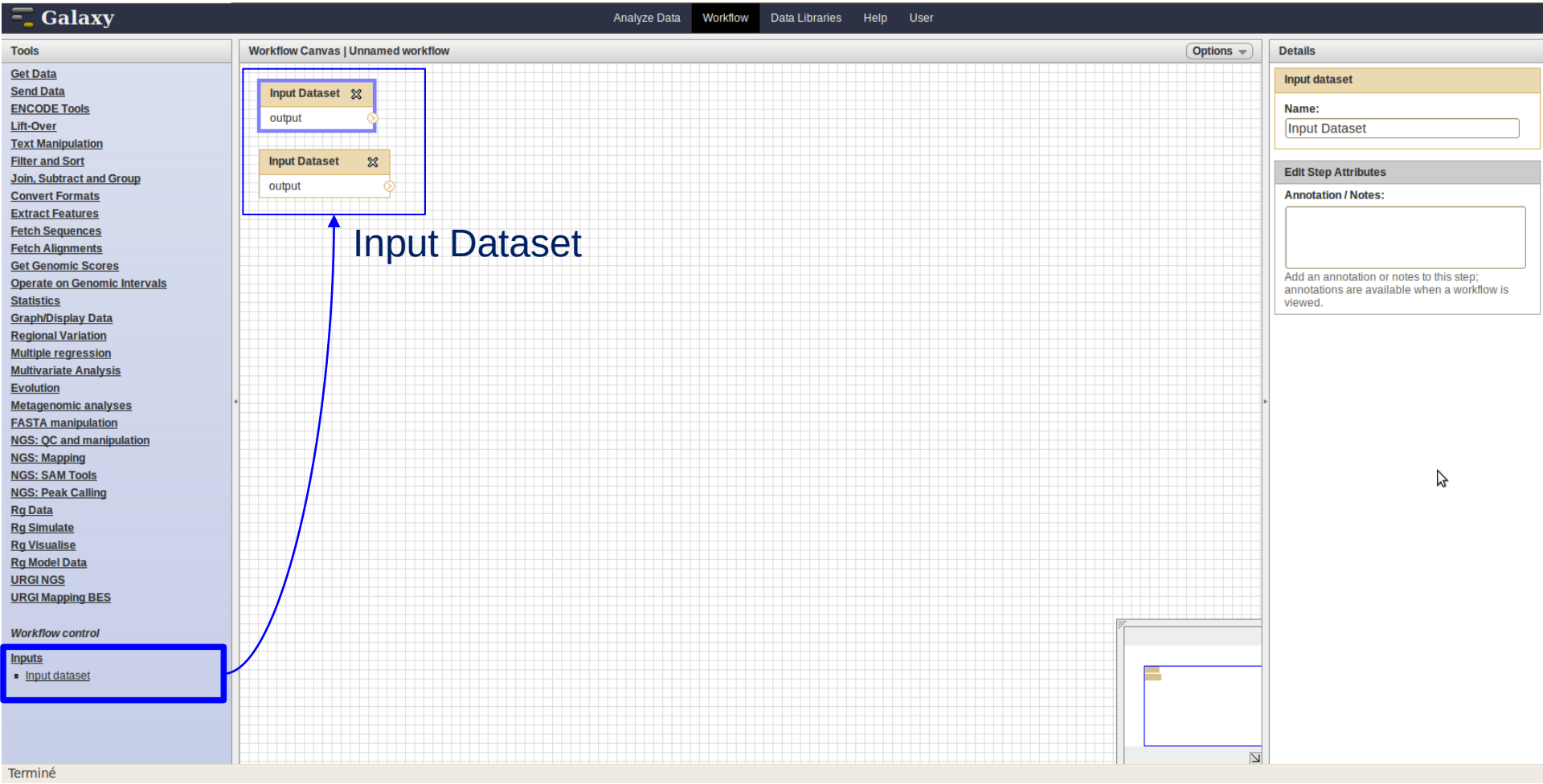

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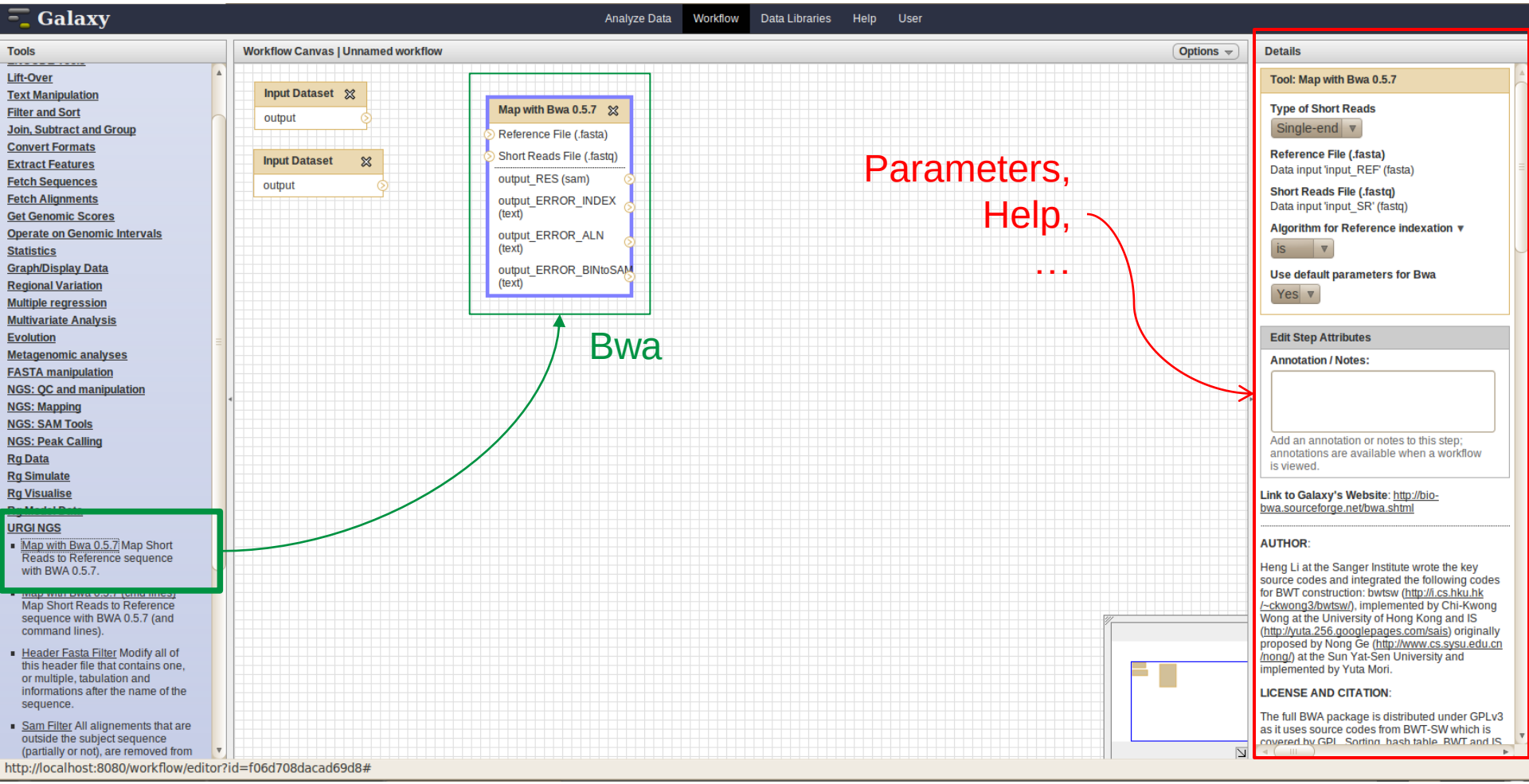

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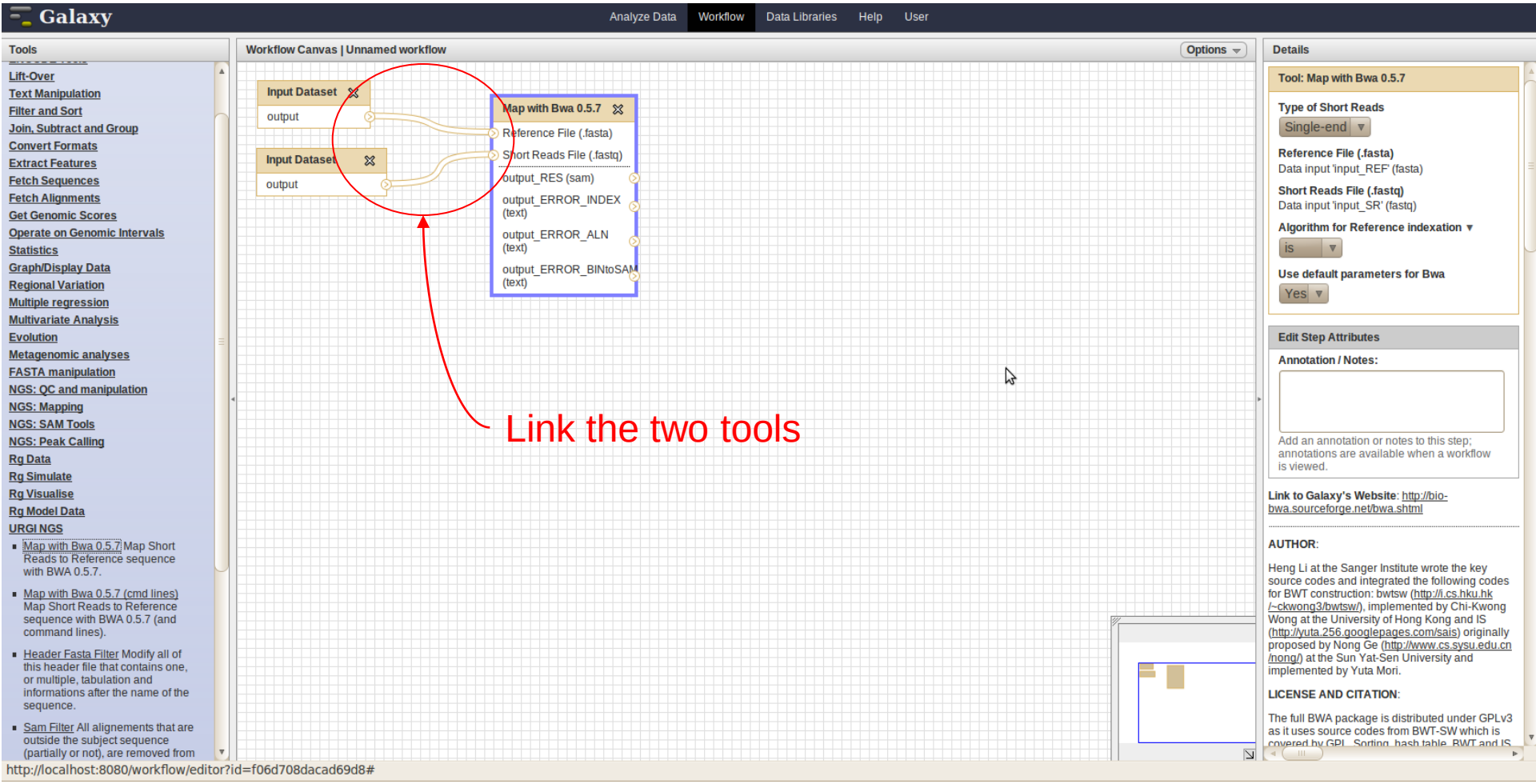

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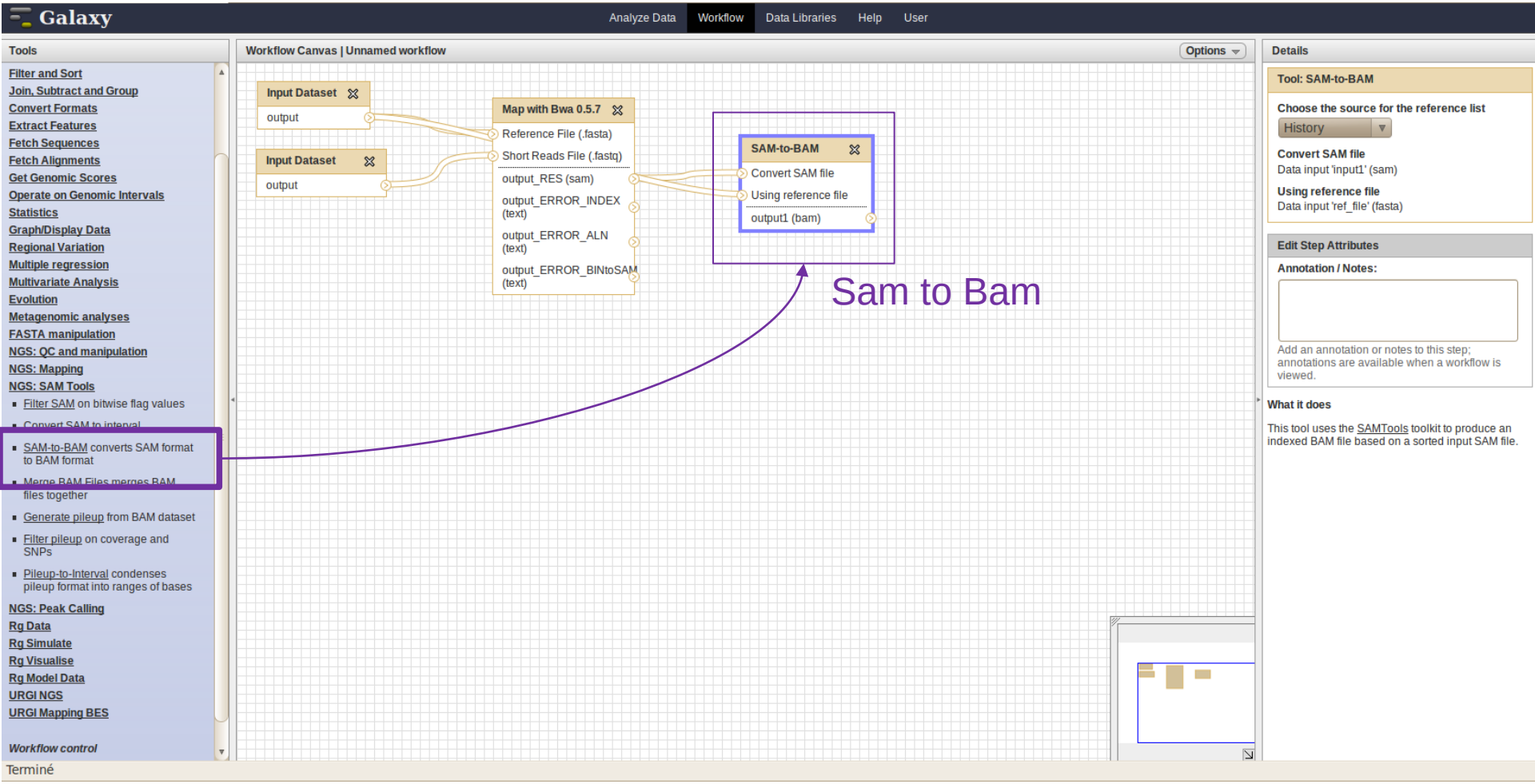

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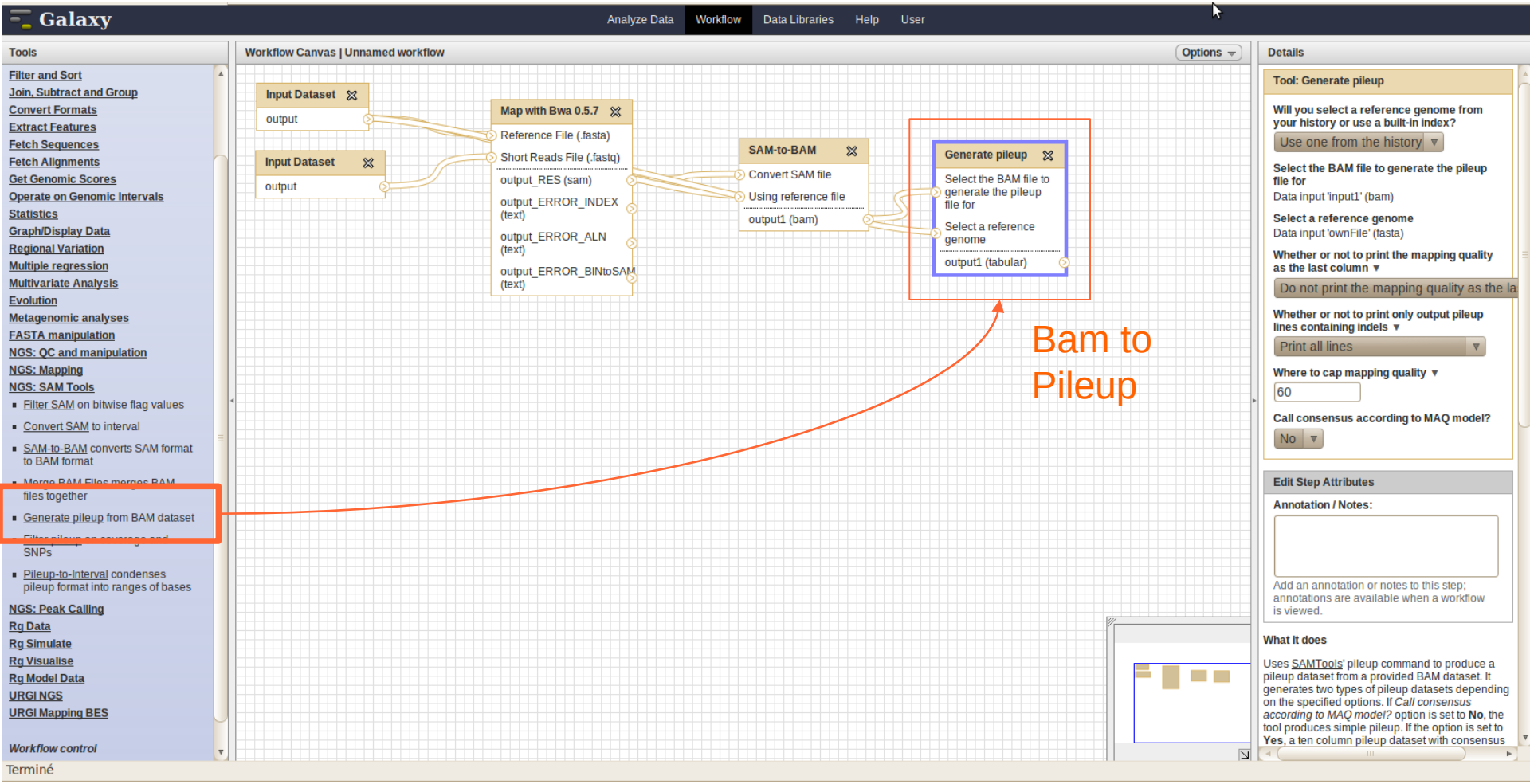

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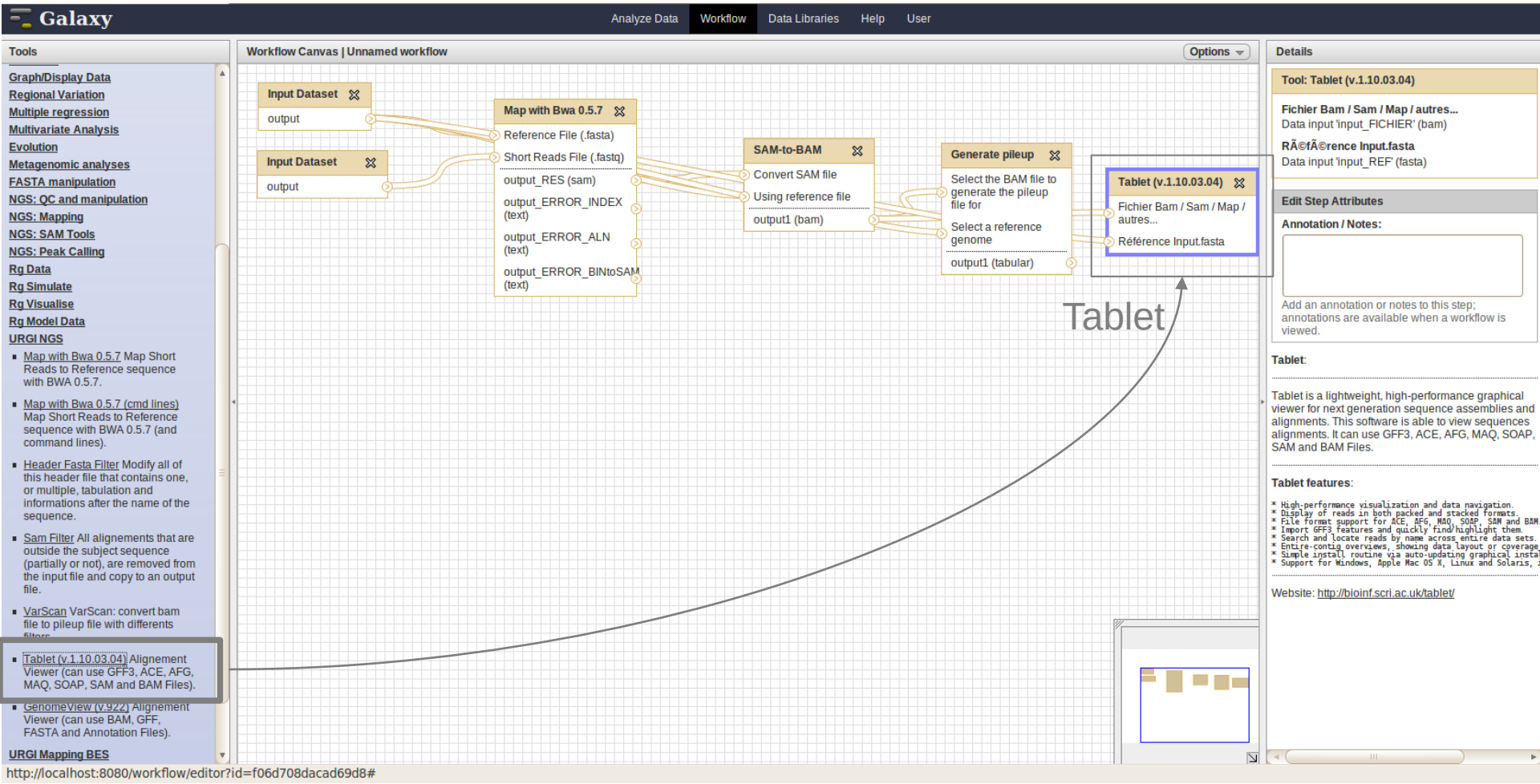

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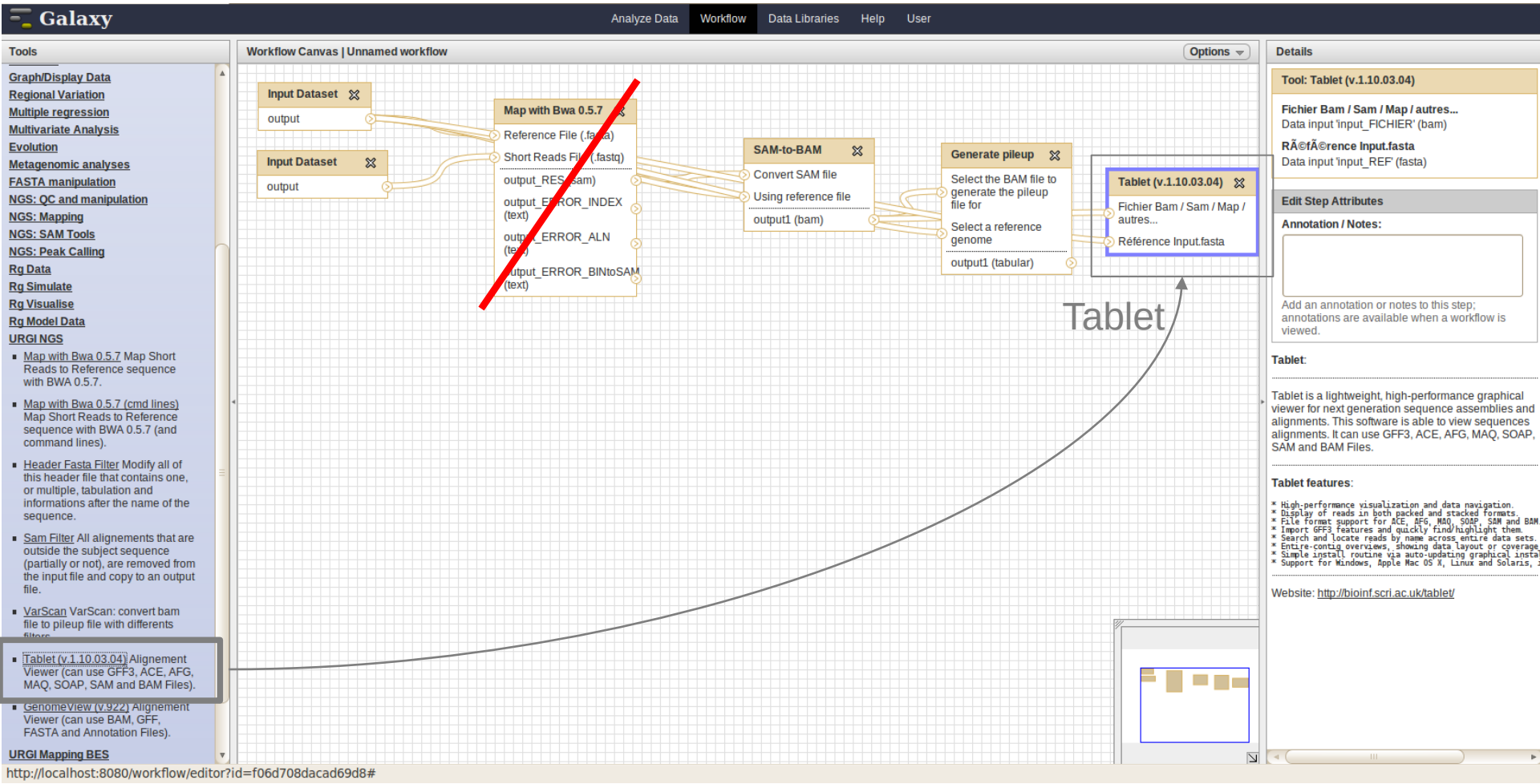

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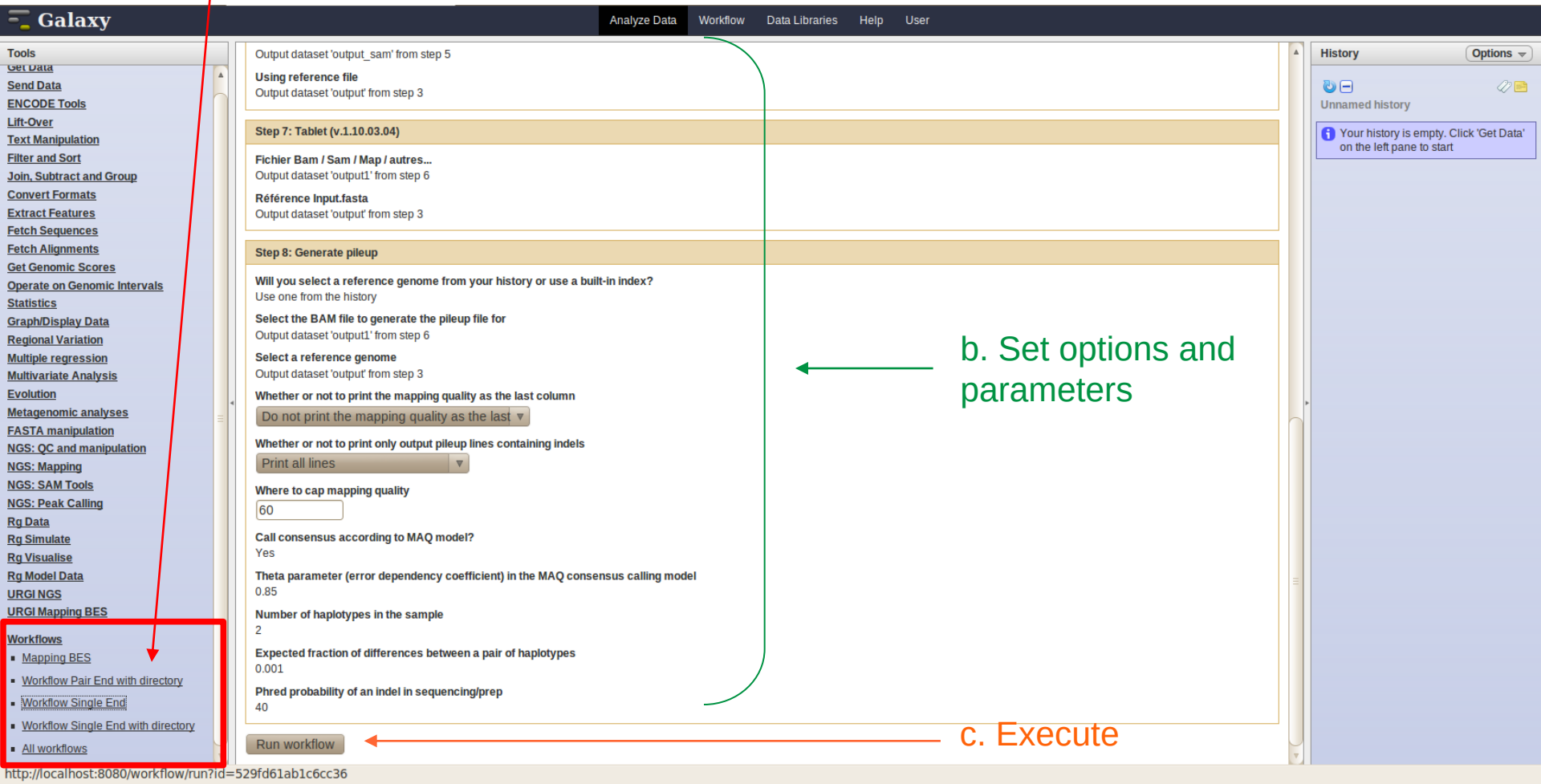
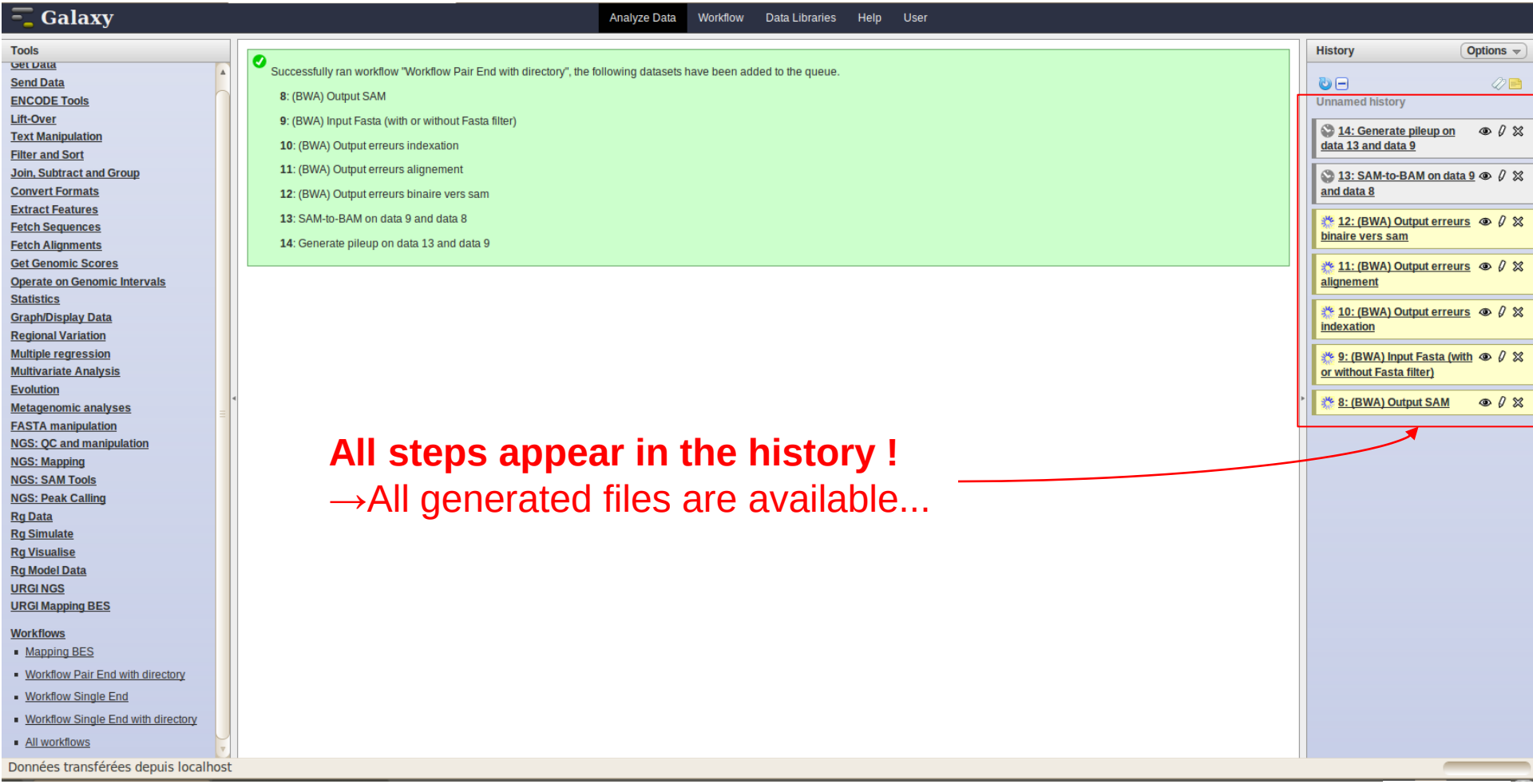
III.6. How to launch the workflow with Galaxy ?

a. Choose the workflow from the list



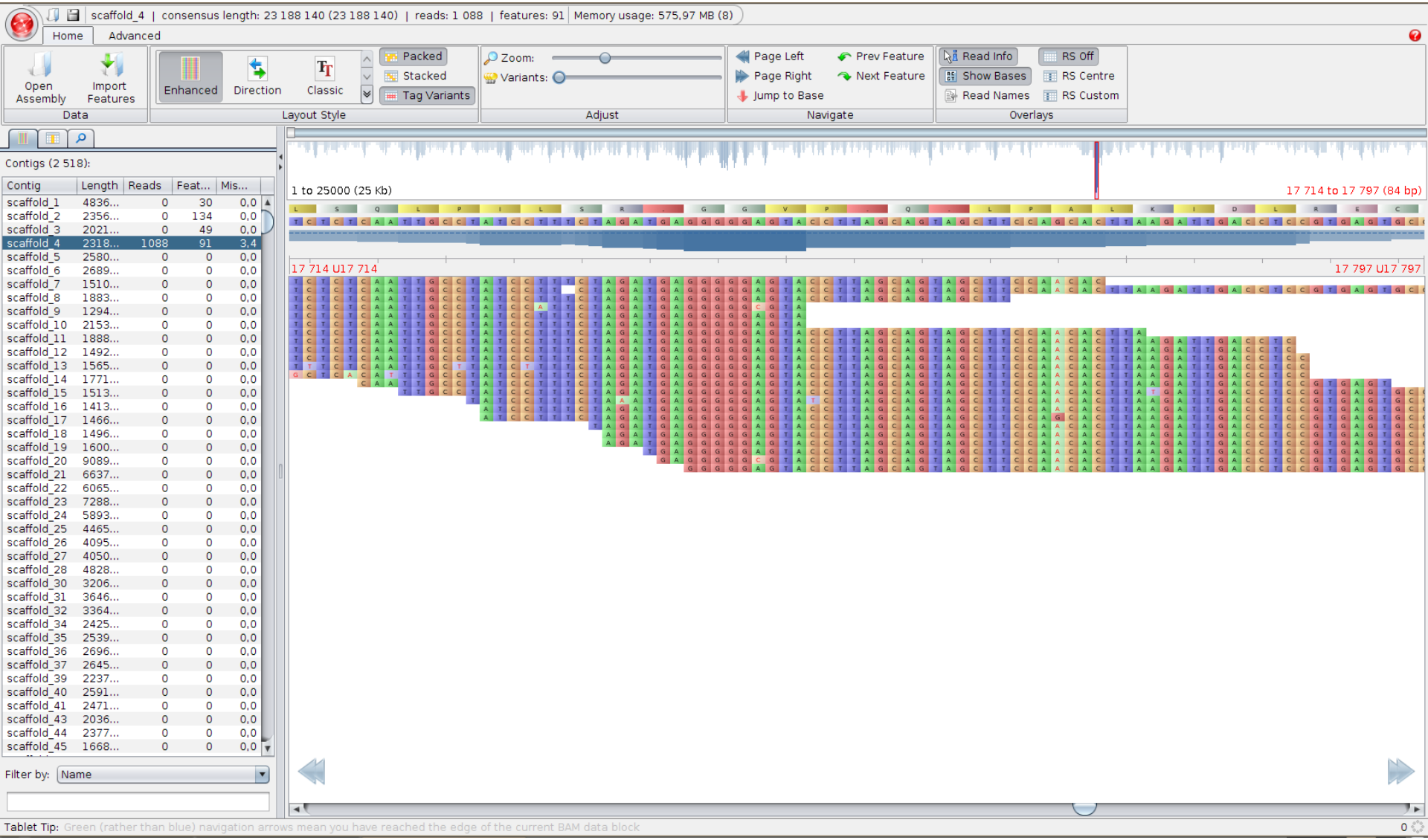
III.6. How to launch the workflow with Galaxy ?



All steps appear in the history !

→All generated files are available...

III.7. Results in Tablet



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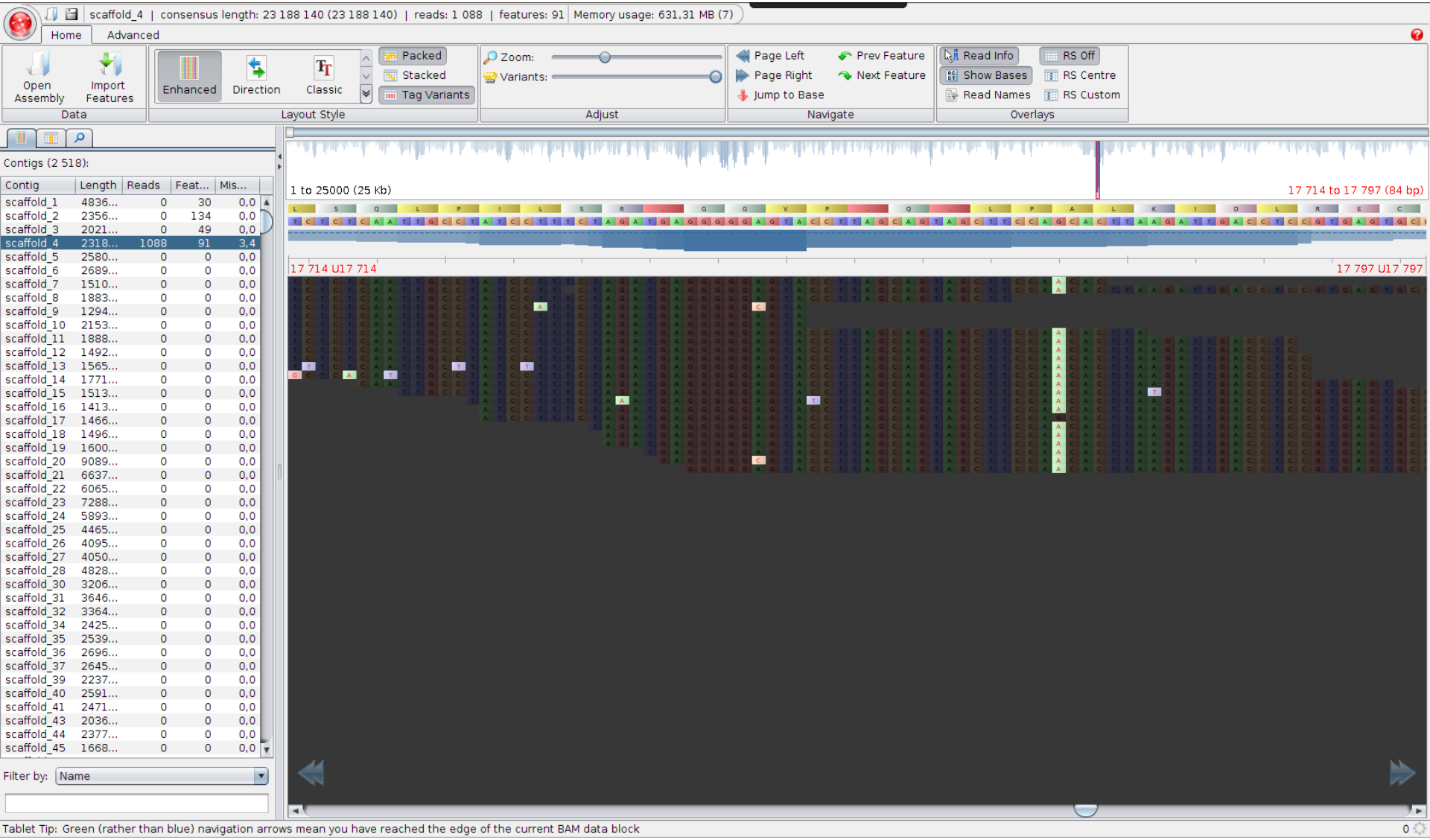
III.7. Results in Tablet



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IV. Validation Process

1. Tests on public data (NCBI → *A. thaliana* and *V. vinifera*).

2. Tests on data from *Tomato* (pair ends, 36 bps) and *Poplar* (pair ends, 70 bps) with comparison of EPGV and GAFL results (project PlanteReseq INRA).

3. Validation / modification of pipeline steps with the help of users.

4. Implementation and final validation of the pipeline with the ANR projects (GrapeReseq and Muscares).

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V. Outlook

- Try other tools for mapping / SNPs calling (according to validation).

- Insert data into our databases:
  → SNPs and Indels in GnpSNP - GnpGenome
  → Contigs and Clusters in GnpSeq

- Put the pipeline on our website !
  → BWA Parallelization on our clusters.

- Visualization of results in Gbrowse.

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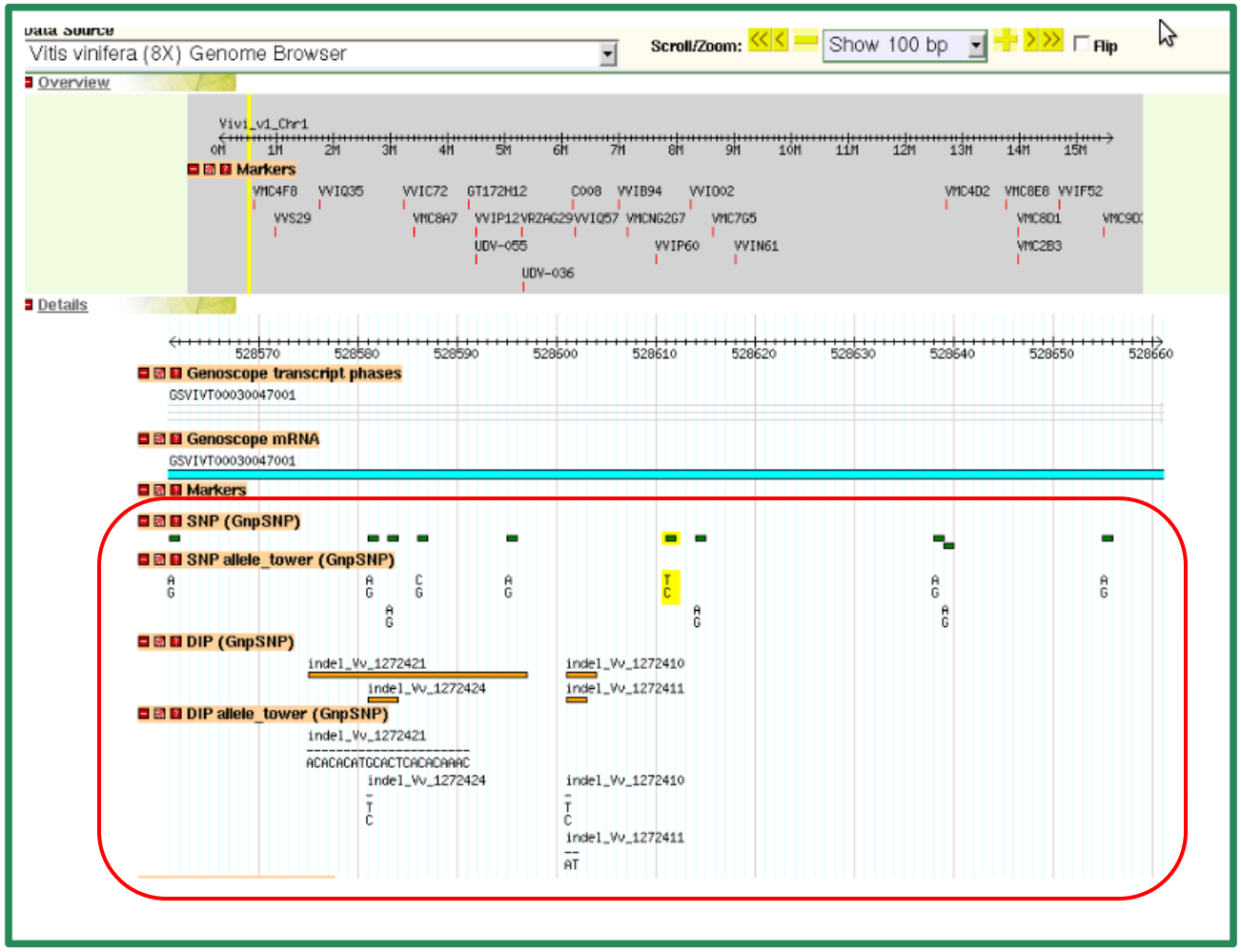
V. Outlook



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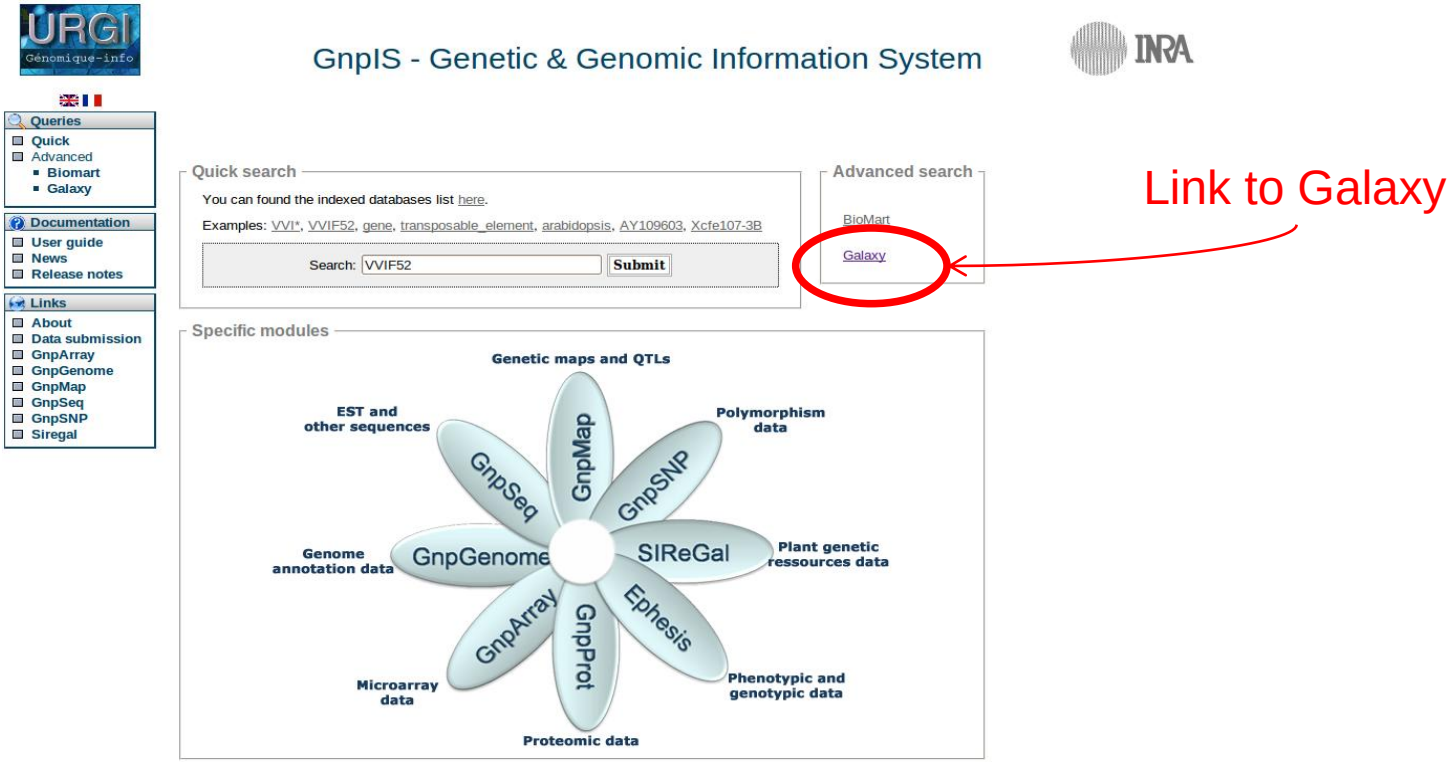
V. Outlook beyond SNP detection

- Interoperability with the BioMart GnpIS URGI server.
  → http://urgi.versailles.inra.fr/gnpis/

- Extension of the system with other tools and pipelines (IBISA 2010 ...).



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URGI	Olivier INIZAN	**EPGV**	Stéphane SCHLUB
URGI	Nacer MOHELLIBI		
URGI	Delphine STEINBACH	**GAFL**	Stéphane MUNOS
URGI	Hadi QUESNEVILLE	**GAFL**	Jean-Paul BOUCHET