

Tools

Options ▾

Your user name: smaman

Your file path : /work/smaman/

1 - UPLOAD YOUR DATA

Get Data

2 - FILES MANIPULATION

Text ManipulationFilter and SortJoin, Subtract and GroupConvert Formats3 - SEQUENCES
MANIPULATIONFASTA manipulationFASTQ manipulationSAM/BAM manipulation : Picard
(beta)SAM/BAM manipulation : SAM
Tools

4 - MAPPING

BWA - Bowtie

5 - INDEL ET SNP

Indel AnalysisRNA-SeqGATK Tools (beta)

6 - SRNASEQ

Analyse des miRNAAnnotationsAlignement sur reference**WELCOME ON SIGENAE GALAXY WORKBENCH**

Galaxy is a workbench available for biologists from Sigenae Platform. Galaxy objectives are:

- Make bioinfo Linux tools accessible to biogists.
 - Hide the complexity of the infrastructure.
- Allow creation, execution and sharing of workflows.

History

Options ▾



TP FastQC

54.0 Mb

8: FastQC_data 5.html **6: GM.fastqsanger** **5: h1.fastqsanger** **4: FastQC_data
18.html** **3: FASTQ Summary
Statistics on data 18** **2: FASTQ Summary
Statistics on data 18**

76 lines, 1 comments
format: tabular, database: ?
Info: 99115 fastq reads were processed.
Based upon quality values and sequence characters, the input data is valid for: sanger
Input ASCII range: '#'(35) - 'C'(67)
Input decimal range: 2 - 34
Epilog : job finished at ven mai 11 10:36:43 CEST 2012



1	2	3	4	5	6
#column	count	min	max	sum	mean
1	99115	2	33	3194703	32.2
2	99115	2	34	3156652	31.8
3	99115	2	34	3145060	31.7
4	99115	2	34	3120431	31.4
5	99115	2	34	3096075	31.2

Durée / Programme : 2 journées et demi.

Galaxy : First step.

Galaxy : Reads alignment and SNP calling.

Public : Personnes souhaitant traiter des données (bio)informatiques sans connaissances spécifiques en informatique (sans avoir à connaître Linux et la ligne de commande).

Liste des sessions disponibles : <http://bioinfo.genotoul.fr>

Les formateurs :

Jour 1 « Initiation »	: Sarah Maman, Ibouniyamine Nabihoudine.
Jour 2 et 3 « SNP »	: Philippe Bardou, Olivier Rué.

Vos traitements bioinformatiques avec GALAXY

Philippe Bardou - Sarah Maman - Ibouniyamine Nabihoudine - Olivier Rué
Avril 2015

<http://galaxy-workbench.toulouse.inra.fr>



Vidéo
disponible sur
« sig-learning »

Présentation de la plateforme Galaxy.

Premiers pas dans l'instance.

Notions d'outils, d'historique et de workflow.

Lancement de traitements bioinformatiques.

Quelques statistiques.

Auto-formations disponibles en ligne.

Equipe “Galaxy project” :

- Le Center for Comparative Genomics and Bioinformatics - Penn State,
- Des départements “Biology” et “Mathematics and Computer Science” de l’Université d’Emory.

Une communauté active autour de cet outil.

Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences

Jeremy Goecks¹, Anton Nekrutenko^{2,3}, James Taylor^{1,3} and The Galaxy Team



EMORY
UNIVERSITY

PENNSTATE



Anton Nekrutenko
Penn State

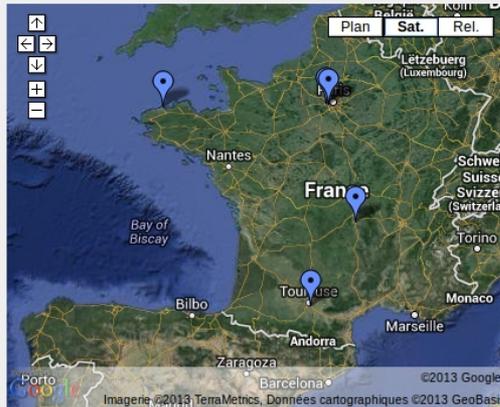


Nate Coraor
Penn State



James Taylor
Emory

- Groupe de travail Galaxy IFB
- Documentation collaborative (wiki)
- Formations (mise en commun agenda PF)
- Architecture
- Intégration d'outils (Tool Shed)
- <http://www.ifb-galaxy.org>



Afficher [Galaxy IFB France](#) sur une carte plus grande

Liste des instances

ABIMS Roscoff	Initiation, NGS Cleaning, RNASeq Differential Expression	http://galaxy.sb-roscoff.fr/	Christophe Caron - Alexandre Cormier - Gildas Lecorguille - Pierre Pericard
Institut Curie	ChIP-Seq Analysis	http://nebula.curie.fr/	Alban Lermine
Genotoul / Sigenae	Initiation to Galaxy, SNP calling, RNASeq, sRNASeq	http://galaxy-workbench.toulouse.inra.fr/	Sarah Maman
INRA URGI	Differential expression analysis, Variant detection	http://urgi.versailles.inra.fr/galaxy2	Olivier Inizan
INRA MIGALE	Initiation to Galaxy, NGS Galaxy	http://migale.jouy.inra.fr/galaxy/	Sandra Derozier - Franck Samson
Southgreen	Generalist platform, and crop breeding	gohelle.cirad.fr/galaxy/root/	Jean-Francois Dufayard
INRA PFEM / MetaboHUB	Metabolomics data analysis	https://pfem-galaxy/	Franck Giacomoni



- Serveur public** (<https://main.g2.bx.psu.edu/>) :
- Gratuit & "open source",
 - Quota limité, petits jeux de données,
 - Impossible d'ajouter des banques, génomes, outils.
 - Données non protégées.

Une communauté nationale et internationale très active

- Listes de diffusion (US, FR)
- Wiki
- Twitter
- "Galaxy tour de France"



L'instance locale Sigenae de Galaxy :

- Maintenu par Sigenae.
 - Intégration possible de nouveaux outils / scripts / génomes ...
- **Présentation des particularités de l'instance Sigenae.**

Inutile de savoir :

- ü Lancer une ligne de commande, un script
- ü Programmer en perl, python, shell ...

Inutile de s'inquiéter pour son disque dur:

- ü Jobs lancés sur un cluster de calculs.
- ü Pas d'archivage de fichiers sur votre PC.

Inutile d'attendre la fin d'un traitement:

- ü Possibilité de lancer plusieurs jobs en parallèle
- ü Partir prendre un café ..fermer votre navigateur! puis voir les résultats le lendemain matin.
- ü

Vous pouvez :

- ü Lancer des traitements depuis votre navigateur,
- ü Dupliquer des traitements,
- ü Partager des analyses complètes,

**Galaxy devient VOTRE BOITE A OUTILS
et ceci de manière très intuitive !**

ü Les limites et règles d'utilisation sont celles de Genotoul

ü L'organisation des données doit être rigoureuse afin de gérer a mieux votre quota

Galaxy est installée sur une machine virtuelle qui envoie les calculs à un cluster



Utilisateur de Galaxy

Envoie de données

Récupération des résultats



Serveur Web Galaxy

Envoie des jobs



Gère la file d'attente

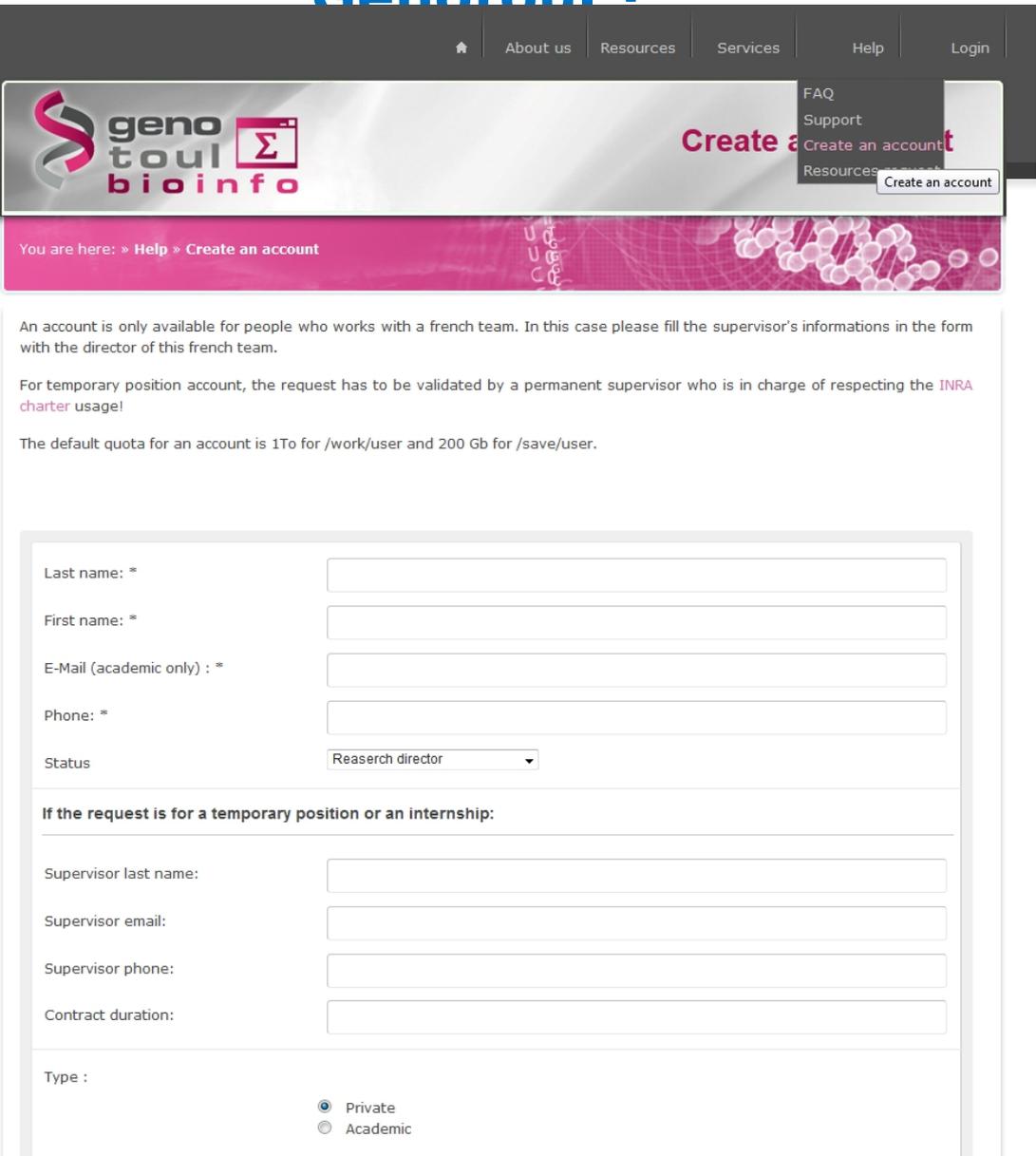
Gestionnaire de tâches

Cluster de calculs



Exécute

Comment ouvrir un compte sur Genotoul ?



The screenshot shows the Genotoul bioinfo website. At the top, there is a navigation menu with links for 'About us', 'Resources', 'Services', 'Help', and 'Login'. A 'Create an account' button is visible in the top right corner. Below the navigation, there is a banner with the Genotoul logo and a 'Create an account' button. A breadcrumb trail indicates the user is in 'Help > Create an account'. The main content area contains text explaining account availability for French teams and temporary positions, and a default quota of 1To for /work/user and 200 Gb for /save/user. Below this is a registration form with the following fields:

- Last name: *
- First name: *
- E-Mail (academic only) : *
- Phone: *
- Status: Reaserch director (dropdown menu)

Below the main form, there is a section for temporary positions or internships with the following fields:

- Supervisor last name:
- Supervisor email:
- Supervisor phone:
- Contract duration:

At the bottom, there is a 'Type' section with radio buttons for 'Private' (selected) and 'Academic'.

1 - Ouvrir un compte sur Genotoul :

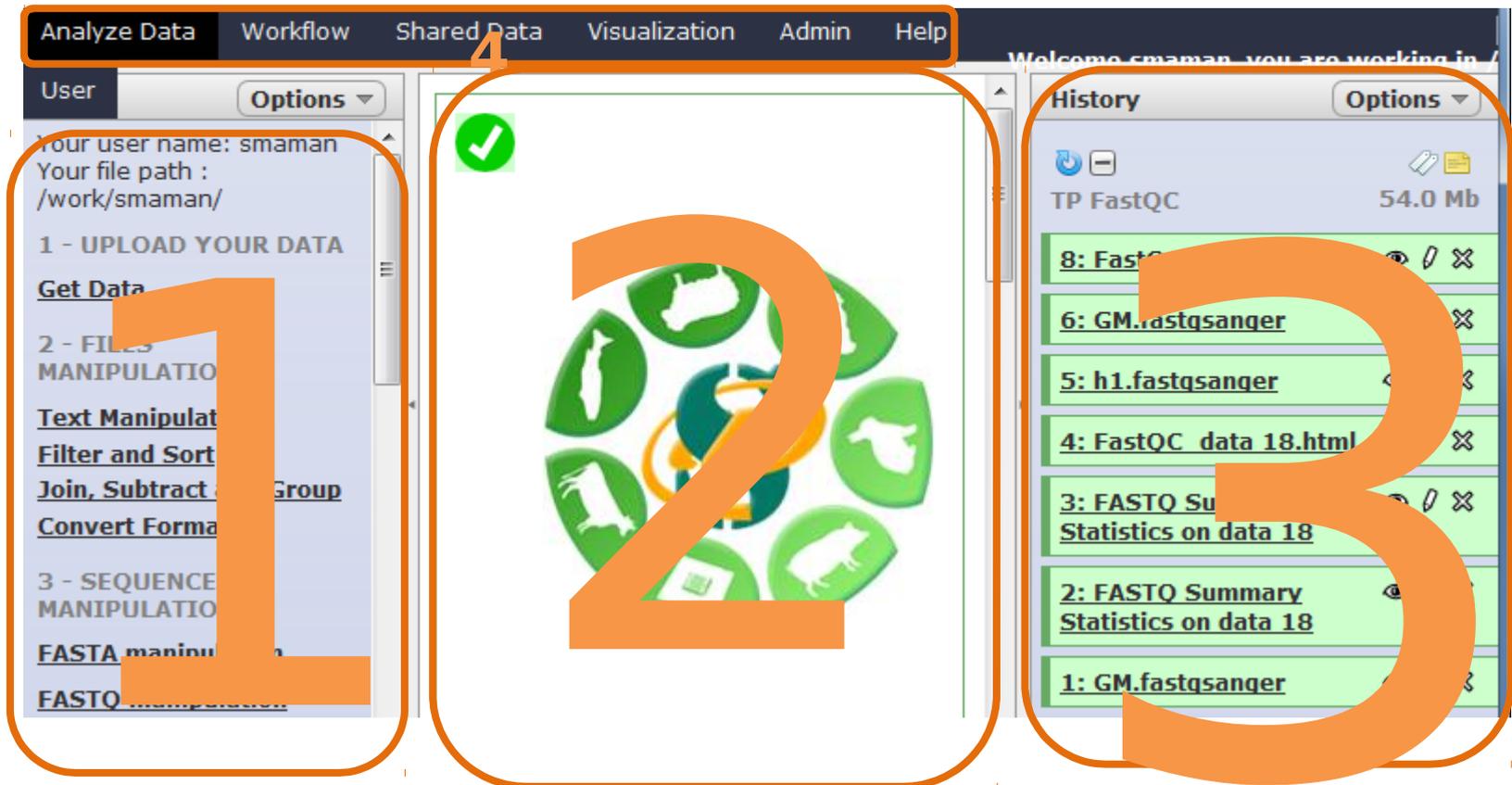
Formulaire de demande de compte:
<http://bioinfo.genotoul.fr>
 (Menu / Help/ Create an account)

2 - Accéder à Galaxy à l'aide du login/mot de passe obtenus :

<http://sigenae-workbench.toulouse.inra.fr/>

Interface divisée en 4 parties :

- 1 - Liste des outils disponibles.
- 2 - Visualisation de l'outil utilisé, historique, dataset ou workflow.
- 3 - Historique ou workflow détaillé.
- 4 - Menu .



Analyze Data Workflow Shared Data Visualization Admin Help Using 13% Welcome smaman, you are working in /work/smaman

User Options ▾

Your user name: smaman
Your file path : /work/smaman/

1 - UPLOAD YOUR DATA

Get Data

2 - FILES MANIPULATION

Text Manipulation
Filter and Sort
Join, Subtract and Group
Convert Formats

3 - SEQUENCES MANIPULATION

FASTA manipulation
FASTQ manipulation
SAM/BAM manipulation : Picard (beta)
SAM/BAM manipulation : SAM Tools

4 - MAPPING

BWA - Bowtie

5 - INDEL ET SNP

Indel Analysis
RNA-Seq
GATK Tools (beta)




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Unnamed history 0 bytes

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*** Upload local file from filesystem path (version 1.0.0)**

File Name:

File type:

Path to file:

History Options

Unnamed history 0 bytes

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Analyze Data Workflow Shared Data Visualization Admin Help

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File Name:

File type:

Path to file:

History Options

Galaxy sensibilisation - TP12.1 Mb
2 - BWA and FastQC

14:
phiX174 reads.fastqsanger
1.0 Mb
format: fastqsanger, database: ?

```
@080917-and-080922:5:1:185:82  
GATGTTATTTCTTCATTTGGAGGTAACCTCTTAT  
+  
IIIIIIIIIIIIIIIIIIII<III@FI8A/I0II4I  
@080917-and-080922:5:1:1366:223  
GTTTTCTTCTGCGTCAGTAAGAACGTCAGTGTTC
```

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NGS: Mapping

- [Lastz map short reads](#) against reference sequence
- [Lastz paired reads](#) map short paired reads against reference sequence
- [Map with Bowtie for Illumina](#)
- [Map with Bowtie for SOLiD](#)
- [Map with BWA for Illumina](#)

BWA - Bowtie

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BWA - Bowtie

5 - INDEL ET SNP

Indel Analysis

RNA-Seq

GATK Tools (beta)

Map with BWA for Illumina (version 1.2.2)

Will you select a reference genome from your l

Use one from the history ▾

Select a reference from history:

11: phiX174_genome.fa ▾

Is this library mate-paired?:

Single-end ▾

FASTQ file:

14: phiX174_reads.fastqsanger ▾

FASTQ with either Sanger-scaled quality values (f

History Options ▾

Unnamed history 0 bytes

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BWA - Bowtie

5 - INDEL ET SNP

Indel Analysis

RNA-Seq

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Will you select a reference genome from your I

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Select a reference from history:

11: phiX174_genome.fa

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Single-end

FASTQ file:

14: phiX174_reads.fastqsanger

FASTQ with either Sanger-scaled quality values (f

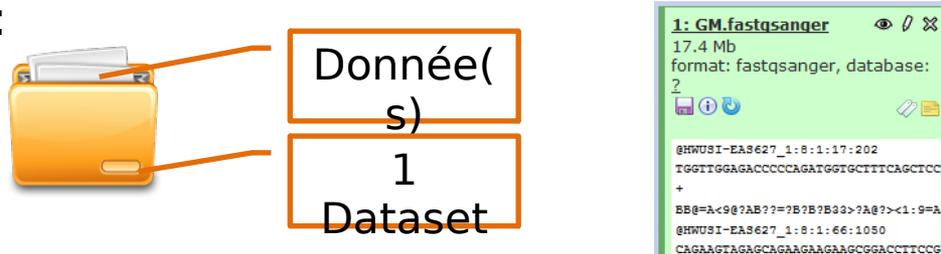
History Options

Unnamed history 0 bytes

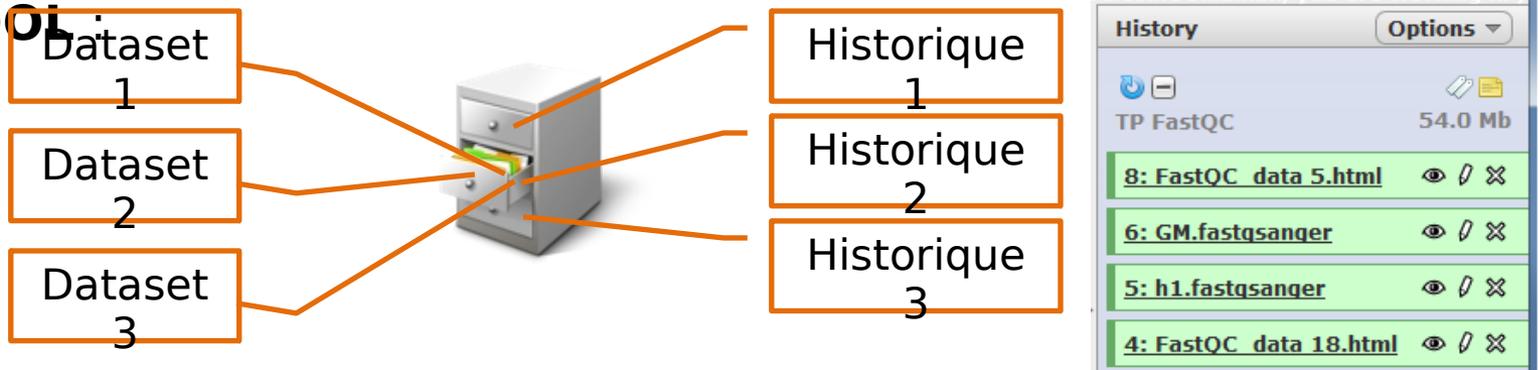
15: Map with BWA for Illumina on data 14 and data 11: mapped reads

Job is waiting to run

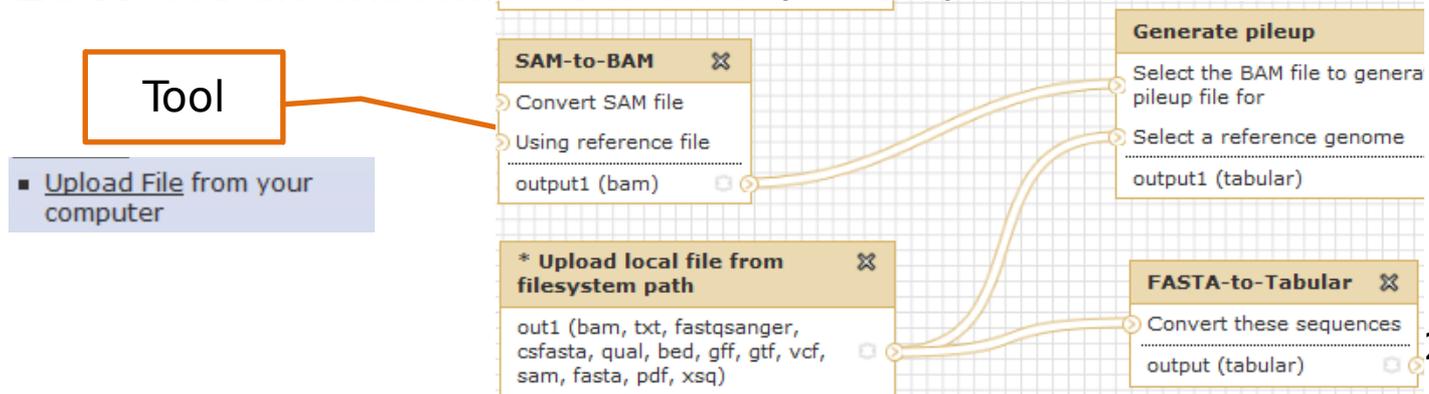
Un **DATASET** est un fichier de données (fichiers d'entrée, fichiers résultats) :



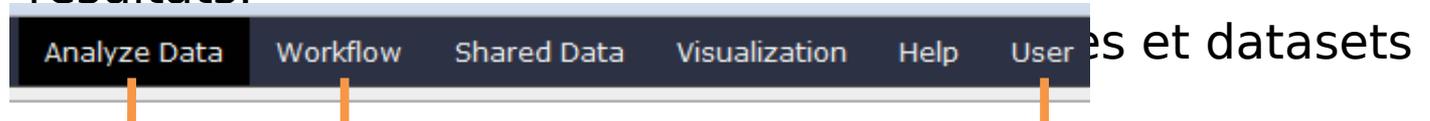
Votre **HISTORIQUE** est un « répertoire » qui « liste » l'ensemble de vos fichiers de données (fichiers d'entrée, fichier résultat) utilisés ou générés par un **TOOL** :



Votre **WORKFLOW** est un ensemble : fichiers, outils, traitements.



- **ANALYSE DATA** : Page d'accueil de Galaxy.
- **WORFLOW** : Liste des workflows .
- **SHARED DATA** : Liste des datasets, historiques et workflows partagés.
- **VISUALIZATION** : Outil de visualisation de vos fichiers résultats.



Your workflows

Name
Workflow constructed from history 'Test BWA fichiers Gnome' ▾
Workflow / 'TP : NGS - Polymorphisme' (imported from uploaded file) ▾
Workflow / 'FastQC' (imported from uploaded file) ▾
Workflow / 'IGV bai' (imported from uploaded file) ▾
Workflow 'Get flanks / region promoters' ▾
Workflow 'RNA seq statistics' et FastQC ▾

Workflows shared with you by others

No workflows have been shared with you.

Saved Histories

search history names and tags

Advanced Search

<input type="checkbox"/> Name	Datasets
<input type="checkbox"/> <u>Unnamed history</u> ▾	
<input type="checkbox"/> <u>Unnamed history</u> ▾	
<input type="checkbox"/> <u>Unnamed history</u> ▾	
<input type="checkbox"/> <u>TP FastQC</u> ▾	7

Présentation de la plateforme Galaxy.

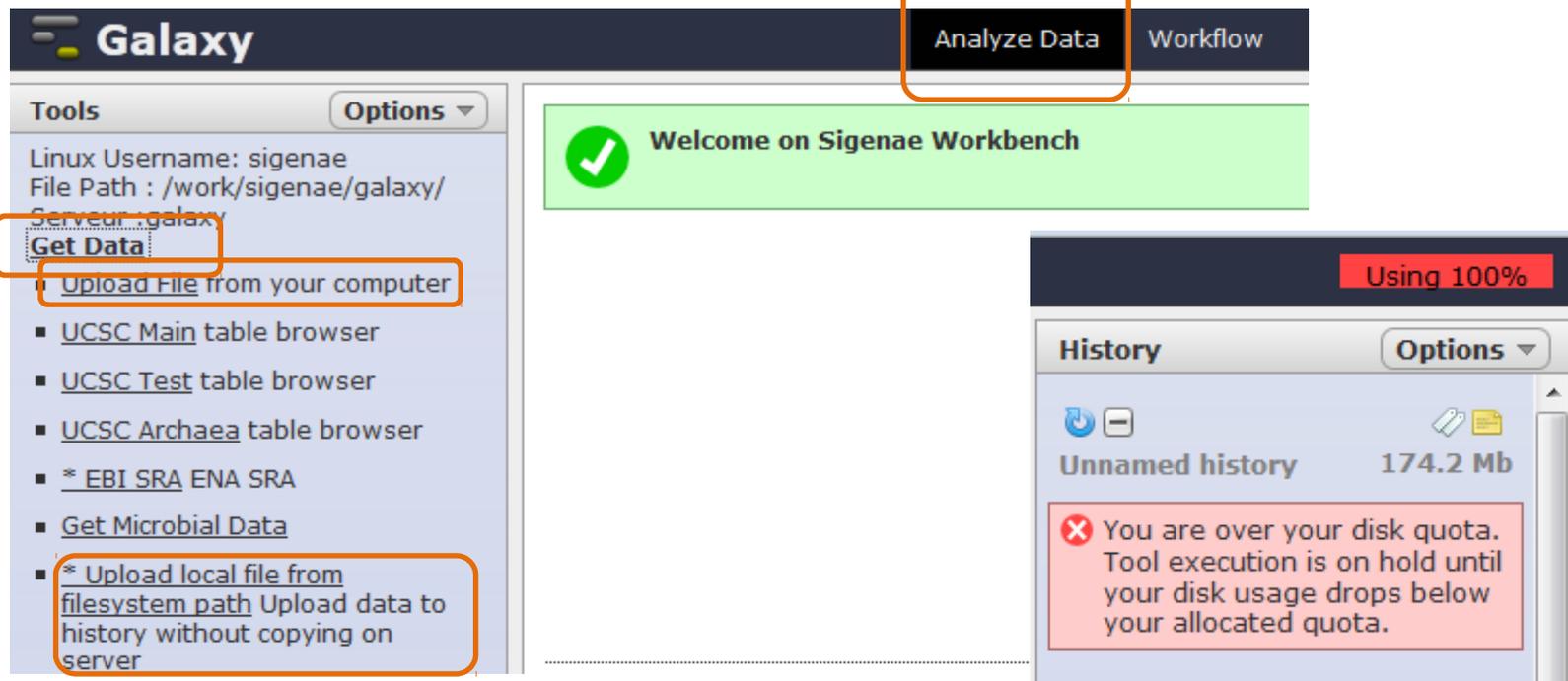
Premiers pas dans l'instance.

Notions d'outils, d'historique et de workflow.

Lancement de traitements bioinformatiques.

Quelques statistiques.

Auto-formations disponibles en ligne.



Solution 1 (recommandée si vos fichiers sont sur Genotoul) :

« Upload local file from filesystem path ».

Solution 2 :

« Upload file from your computer, with a copy on server ».

 L'espace disque utilisé n'est pas celui de votre PC.
 Vos traitements ne sont pas limités par la capacité de votre PC.

NG6 (Next Generation Sequencing Information System) :

- Environnement de stockage et de mise à disposition des données issues des nouvelles technologies de séquençage.
- Organisé autour des notions de projet et de run, permet un accès sécurisé aux données brutes, aux statistiques de traitements ainsi qu'aux assemblages et annotations produites.

<http://ng6.toulouse.inra.fr>



- Project Demonstration
- Project Demonstration2
- Project Galaxy training
- Run Galaxy - First steps (Sample) - (25-10-13) produced 10000 reads
 - Raw data
 - Analyse Data files

User login

Enter your username and password here in order to log in on the website:

Login

Username:

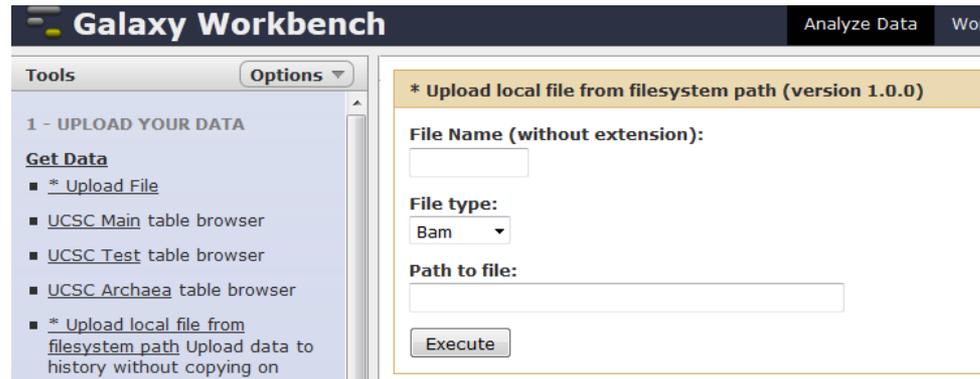
Password:

Keep up with news

- June 17 2013** NG6 v2.0 is now available. This new version is based upon the jflow workflow engine instead of ergatis. This version is coming with several new features for runs administrators.
- November 27 2012** NG6 is now only available in english.
- September 14 2012** New user management system. 3 rights levels: administrator (in charge to run workflows), manager (in charge to manage project access) and member (browsing projects/runs/analyses).
- September 9 2012** Publication of NG6 in [BMC Genomics](#).
- August 7 2012** NG6 is available in french and in english.
- June 4 2012** Fix a bug in the generation of cigarline graphs produced by the AlignmentStats analysis.
- November 23 2011** Since the 3rd of october 2011 the HiSeq quality encoding is in Sanger format, no longer in illumina format.
- August 2nd 2011** New functionalities are available to browse your projects/runs /analyses. A hierarchical display is now available for analysis. For project administrators new functionalities are also available.

-Soit fichier par fichier (repris en TP) :

-
-
-
-
-
-



Galaxy Workbench Analyze Data Wor

Tools Options

1 - UPLOAD YOUR DATA

Get Data

- * Upload File
- UCSC Main table browser
- UCSC Test table browser
- UCSC Archaea table browser
- * Upload local file from filesystem path Upload data to history without copying on

*** Upload local file from filesystem path (version 1.0.0)**

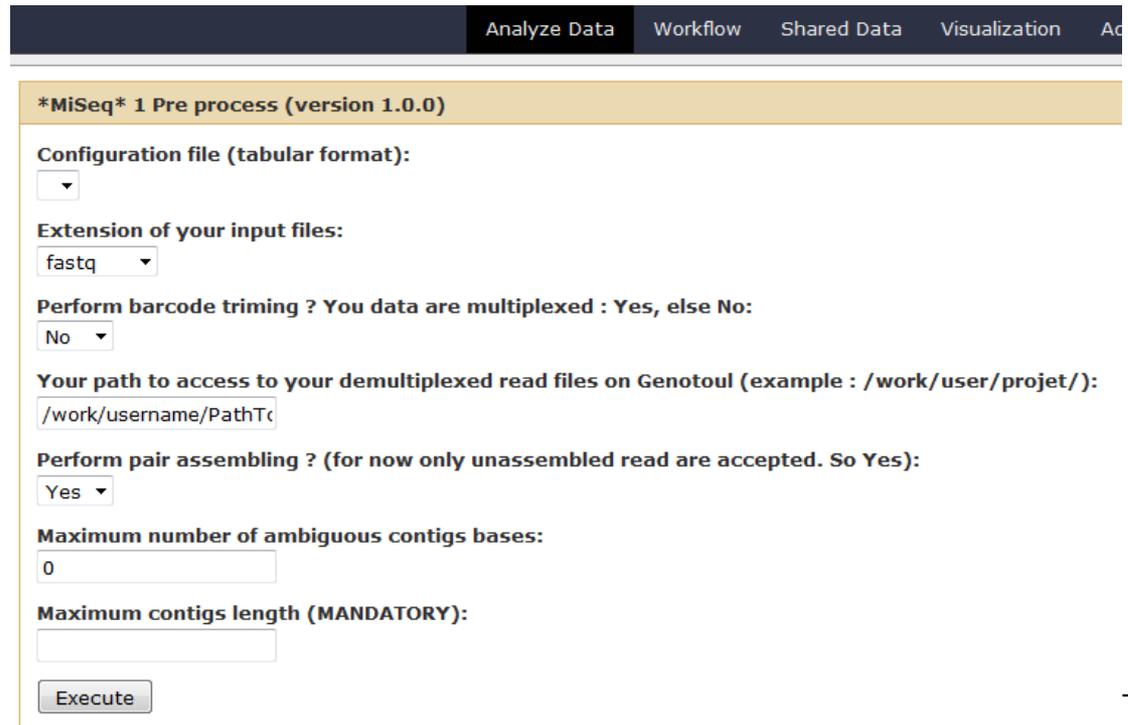
File Name (without extension):

File type:
 Bam

Path to file:

Execute

-Soit un répertoire de fichiers :



Analyze Data Workflow Shared Data Visualization Ac

***MiSeq* 1 Pre process (version 1.0.0)**

Configuration file (tabular format):

Extension of your input files:
 fastq

Perform barcode trimming ? You data are multiplexed : Yes, else No:
 No

Your path to access to your demultiplexed read files on Genotoul (example : /work/user/projet/):
 /work/username/PathTc

Perform pair assembling ? (for now only unassembled read are accepted. So Yes):
 Yes

Maximum number of ambiguous contigs bases:

Maximum contigs length (MANDATORY):

Execute



Données UCSC, Ensembl, BIOMART :

clade: Mammal genome: Human assembly: Feb. 2009 (GRCh37/hg19)

group: Genes and Gene Prediction Tracks track: UCSC Genes

table: knownGene

region: genome

identifiers (names/accessions):

filter: create

intersection: create

correlation: create

output format: BED - browser extensible data

output file: (leave blank to keep output in browser)

file type returned: plain text

Galaxy

Analyze Data Workflow Shared Data Admin Help User

EMBL-EBI

European Nucleotide Archive

Text search

Enter search query, for example: BN000065

bio.mart

New Count Results

Dataset

Exercice 1 :

Connexion à Galaxy, exploration de l'interface,
téléchargement de datasets

Présentation de la plateforme Galaxy.

Premiers pas dans l'instance.

Notions d'outils, d'historique et de workflow.

Lancement de traitements bioinformatiques.

Quelques statistiques.

Auto-formations disponibles en ligne.

History Options ▾

TP FastQC 54.0 Mb

- 8: [FastQC_data 5.html](#) 👁️ ✂️
- 6: [GM.fastqsanger](#) 👁️ ✂️
- 5: [h1.fastqsanger](#) 👁️ ✂️
- 4: [FastQC_data 18.html](#) 👁️ ✂️
- 3: [FASTQ Summary Statistics on data 18](#) 👁️ ✂️
- 2: [FASTQ Summary Statistics on data 18](#)** 👁️ ✂️
 76 lines, 1 comments
 format: tabular, database: ?
 Info: 99115 fastq reads were processed.
 Based upon quality values and sequence characters, the input data is valid for: sanger
 Input ASCII range: '#'(35) - 'C'(67)
 Input decimal range: 2 - 34
 Epilog : job finished at ven mai 11 10:36:43 CEST 2012
📄 ⓘ 🔄

1	2	3	4	5	6
#column	count	min	max	sum	mean
1	99115	2	33	3194703	32.2
2	99115	2	34	3156652	31.8
3	99115	2	34	3145060	31.7

- Conserver toutes les étapes de vos analyses.
-
-
- Partager vos analyses.
-
-
- A chaque (re)run d'un outil, un nouveau dataset est créé. Les données ne sont pas écrasées.
-
- Répéter, autant de fois que nécessaire, une analyse en modifiant vos paramètres pour explorer les différences de résultats.

<input type="checkbox"/>	SwanPorc ▾	18		0 Tags	Shared	0 bytes
<input type="checkbox"/>	FastQC ▾	6		0 Tags	Shared	17.4 Mb
<input type="checkbox"/>	TP : NGS - Polymorphisme ▾	8	2	0 Tags	Shared	6.6 Gb
<input type="checkbox"/>	TP FastQC ▾	12	16	0 Tags		54.0 Mb
<input type="checkbox"/>	indexation genome ▾	1		0 Tags		46 bytes
For 0 selected histories: Rename Delete Delete Permanently						

Historiques sauvegardés, publiés et partagés

The screenshot shows the Galaxy Sig interface. At the top, there are navigation tabs: 'Galaxy Sig', 'Analyze Data', 'Workflow', 'Shared Data', and 'Visual'. Below these, there's a search bar for 'Published Histories' with the placeholder text 'search name, annotation, owner, and tags'. A table lists published histories, with one entry: 'TP 1 suite : region promotrices' by user 'smaman'. To the right, a dropdown menu is open, showing options like 'Data Libraries', 'Published Histories', 'Published Workflow', and 'Published Visualizations'. Another window titled 'Histories shared with you by others' is visible, showing a list of shared histories.

• Vos historiques sont automatiquement sauvegardés.
 Pour partager ou publier un historique :
 User / Saved histories / Cliquer sur le nom de l'historique / Share ou Publish

-
-
-
-
-
-
-

This screenshot shows the user menu for 'smaman' in the Galaxy interface. The user is logged in as 'smaman@toulouse.inra.fr'. The menu options include 'Logout', 'Saved Histories', 'Saved Datasets', and 'Public Name'. The background shows a table with columns for 'Datasets' and 'Tags', with some data visible.

• Vos historiques publiés sont accessibles à l'ensemble des utilisateurs loggés sur Galaxy (Shared Data / Published Histories).
 • Les historiques partagés sont accessibles uniquement à un utilisateur spécifique (History / Option / Histories Shared With Me).

Comment lancer un job sans ligne de commande ?

1 - Télécharger vos données :

* Upload local file from filesystem path (version 1.0.0)

File Name:

File type:

Path to file:

Galaxy sensibilisation - TP 12.1 Mb
 2 - BWA and FastQC

14:
phiX174_reads.fastqsanger
 1.0 Mb
 format: fastqsanger, database: ?

```
@080917-and-080922:5:1:185:82
GATGTTATTTCTTCATTGGAGGTAACCTCTTAT
+
IIIIIIIIIIIIIIIIIIII<III@FI8A/I0II4I
@080917-and-080922:5:1:1366:223
GTTTCTTCTGCGTCAGTAAGAACGTCAGTGTTC
```

11: phiX174_genome.fa
 1 sequences
 format: fasta, database: ?
 Info: uploaded fasta file sur :
 ftp://ftp.gmod.org/pub/gmod
 /Courses
 /2010/SummerSchoolAmericas
 /Galaxy/phiX174_genome.fa

```
>phix
GAGTTTTATCGCTTCCATGACGCAGAAGTTAACCTT
AAATTATCTTGATAAAGCAGGAATTACTACTGCTTGT
TGCTGGCGGAAAATGAGAAAATTCGACCTATCCTTGC
GCGACCTTTCGCCATCACTAACGATTCTGTCAAAA
TGGCTTAATATGCTTGGCAGCTTCGTCAAGGACTGGT
```

2 - Choisir un outil dans « Tools » :

NGS: Mapping

- [Lastz map short reads](#) against reference sequence
- [Lastz paired reads](#) map short paired reads against reference sequence
- [Map with Bowtie for Illumina](#)
- [Map with Bowtie for SOLiD](#)
- [Map with BWA for Illumina](#)

Map with BWA for Illumina (version 1.2.2)

Will you select a reference genome from your I

Select a reference from history:

Is this library mate-paired?:

FASTQ file:

FASTQ with either Sanger-scaled quality values (f

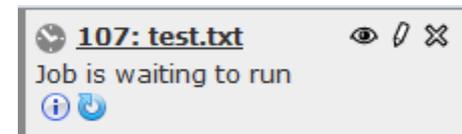
3 - Lancer le job en cliquant sur « Executer ».

L'execution du job en cours est visible dans votre historique.

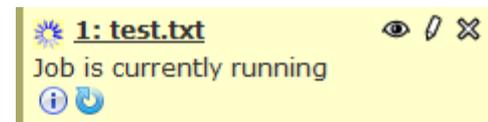
15: Map with BWA for
Illumina on data 14 and data 11:
mapped reads
 Job is waiting to run

Fini les lignes de commande !

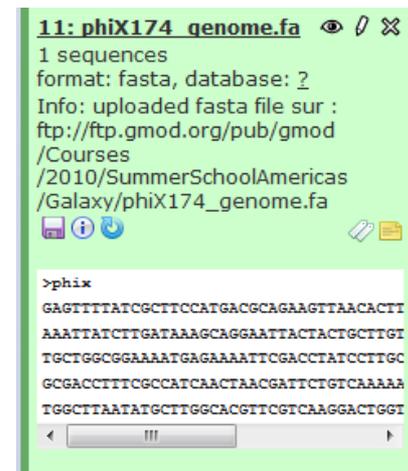
Etat 1 - GRIS : Votre job est en file d'attente.



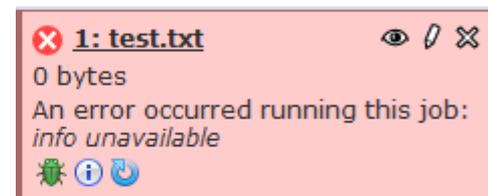
Etat 2 - JAUNE : Votre job est en cours d'exécution



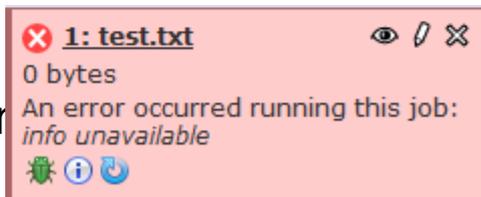
Etat 3 - VERT : Votre job est fini.



Bug - ROUGE : Votre job est planté !



Bug - **ROUGE** : Votre job est plané



Voici les informations à transmettre par mail à sigeane-support@listes.inra.fr :

- Le contenu (copier/coller) du bug ou/et (i)/stderr
- Un share de votre historique.

Dataset generation errors

Dataset 3: ContigLengthG1000ProfG8.res

Tool execution generated the following error message:

```

Traceback (most recent call last):
  File "/usr/local/bioinfo/src/galaxy/galaxy-dist/tools/sm_clones/scripts_module2/ace_statistics.py", line 98, in <module>
    pit.clf()
  File "/usr/local/bioinfo/src/python/current/lib/python2.7/site-packages/matplotlib/pyplot.py", line 443, in clf
    gcf().clf()
  File "/usr/local/bioinfo/src/python/current/lib/python2.7/site-packages/matplotlib/pyplot.py", line 369, in gcf
    return figure()
  File "/usr/local/bioinfo/src/python/current/lib/python2.7/site-packages/matplotlib/pyplot.py", line 343, in figure
    **kwargs)
  File "/usr/local/bioinfo/src/python/current/lib/python2.7/site-packages/matplotlib/backends/backend_tkagg.py", line 80, in new_figure_manager
    window = Tk.Tk()
  File "/usr/local/bioinfo/src/python/current/lib/python2.7/lib-tk/Tkinter.py", line 1685, in __init__
    self.tk = tkinter.create(screenName, baseName, className, interactive, wantobjects, useTk, sync, use)
tkinter.TclError: no display name and no $DISPLAY environment variable
    
```

The tool produced the following additional output:

```

Votre repertoire de travail : /work/galaxy/database/files/workspace/38401
Epilog : job finished at mar. avril 1 15:47:29 CEST 2014
    
```

Report this error to Sigeneae Team

Please create a ticket in [Redmine](#) or send a mail to [Galaxy administrator](#).

Tool: Clone metagenomic

Name:	ContigLengthG1000ProfG8.res
Created:	Apr 01, 2014
Filesize:	134 bytes
Dbkey:	?
Format:	txt
Tool Version:	
Tool Standard Output:	stderr
Tool Standard Error:	stderr

Input Parameter

Other ace files
Other ace files

Inheritance Chain

ContigLengthG1000ProfG8.res

Vous pouvez aussi créer un ticket sous Redmine.

Galaxy Sig Analyze Data Workflow Shared Data Visualization Admin Help User Welcome smaman Using 30%

Tools Options

1 - UPLOAD YOUR DATA

Get Data

2 - FILES MANIPULATION

Text Manipulation

Filter and Sort

Join, Subtract and Group

Convert Formats

3 - SEQUENCES MANIPULATION

FASTA manipulation

FASTQ manipulation

SAM/BAM manipulation : Picard (beta)

SAM/BAM manipulation : SAMtools

Fetch Sequences

Saved Histories

search history names and tags

Advanced Search

Name	Datasets	Tags	Sharing
TP Galaxy project	2	1	0 Tags
miRNA tests	59	21	0 Tags
TP SNPs calling	84	9	0 Tags
TP RNAseq	88	1	0 Tags Shared, Accessible
test TP miRNA	36	1	0 Tags
Unnamed history			0 Tags

Analyse OK

Analyse en attente

Analyse en erreur

Analyse en cours

2: UCSC Main on Human: snp137Common (chr22:1-51304566) ~180,000 regions format: bed, database: hg19 view in GeneTrack display at Ensembl Current



Rapidement, beaucoup de données sont générées

→ D'où l'importance de bien nommer ses historiques / datasets / workflows pour les trier et les organiser au mieux.

Galaxy Workbench
Using 37%

Analyze Data
Workflow
Shared Data
Visualization
Admin
Help
User
Welcome smaman

Tools

FASTA manipulation

FASTQ manipulation

SAM/BAM manipulation : Picard (beta)

SAM/BAM manipulation : SAMtools

Fetch Sequences

Sequences Queries

4 - SGS MAPPING

BWA - Bowtie

5 - SNP / INDEL

GATK Tools (beta)

SAMtools

Indel Analysis

6 - TRANSCRIPTOMIC

RNAseq

RNASEQ ALIGNEMENT

- **Tophat for Illumina** Find splice junctions using RNA-seq data
- htseq count
- Merge Htseq count output file into a global counting file
- Slicufflinks to obtain raw count of reads
- Merge slicufflinks count file

RNASEQ RAW EXPRESSION

7 - CHIP-SEQ

Operate on Genomic Intervals

Nebula

8 - TRAININGS

Galaxy Initiation

Reads alignment and SNP calling

RNA-Seq

sRNAseq

SNP annotation

*** EN COURS DE TEST * Tophat for Illumina (version 1.0.0)**

Your RNA-Seq FASTQ file (read 1):
2: M_Pf_2_ACAGTG_L00..fastqsanger

Your RNA-Seq FASTQ file (read 2):
2: M_Pf_2_ACAGTG_L00..fastqsanger

Your RNA-seq FASTQ file are zipped:
 Yes
Please check this option if your files are zipped.

Choose your reference genome:
Select a reference genome
Please choose either use a bank available on your BioInfo Genotoul Plateform or use your own FASTA reference file (this FASTA file will automatically be indexed by Galaxy)

Select a reference genome:
Danio rerio Zv9 62 chr 22

Number of threads used to align reads:
16

Maximum intron length:
5000

Expected (mean) inner distance between mate pairs:
200

More options ?:
No more option
Please choose Show if you want to see more options.

What is Tophat?

reads to a genome in order to identify exon-exon splice junctions. It is built on the ultrafast short read mapping program Bowtie. TopHat

TopHat was designed to work with reads produced by the Illumina Genome Analyzer, although users have been successful in using TopHat with reads from other technologies. In 1.1.0, we began supporting Applied Biosystems' Colospace format. The software is optimized for reads 75bp or longer.

Mixing paired- and single- end reads together is not supported.

How does TopHat find junctions?

TopHat can find splice junctions without a reference annotation. By first mapping RNA-Seq reads to the genome, TopHat identifies potential exons, since many RNA-Seq reads will contiguously align to the genome. Using this initial mapping information, TopHat builds a database of possible splice junctions and then maps the reads against these junctions to them.

Short read sequencing machines can currently produce reads 100bp or longer but many exons are shorter than this so they would be missed in the initial mapping. TopHat solves this problem mainly by splitting all input reads into smaller segments which are then mapped independently. The segment alignments are put back together in a final step of the program to produce the end-to-end read alignments.

TopHat generates its database of possible splice junctions from two sources of evidence. The first and strongest source of evidence for a splice junction is when two segments from the same read (for reads of at least 45bp) are mapped at a certain distance on the same genomic sequence or when an internal segment fails to map - again suggesting that such reads are spanning multiple exons. With this approach, "GT-AG", "GC-AG" and "AT-AC" introns will be found ab initio. The second source is pairings of "coverage islands", which are distinct regions of

History

Test / Phylofish 3.9 Gb

15:
{(M Pf 2 ACAGTG L007 R2.fastqsanger)-BWA.bam
}-head.txt
100 lines
format: txt, database: 2
Info: Epilog : job finished at mar. juil. 16 11:35:24 CEST 2013

8BQ	VM:1.3	80:coordinate		
8BQ	SM: ENSDART00000112989	LN: 2064		
8BQ	SM: ENSDART00000122537	LN: 579		
8BQ	SM: ENSDART00000129800	LN: 810		
8BQ	SM: ENSDART00000099219	LN: 2553		
8BQ	SM: ENSDART00000079354	LN: 1245		

14:
{(M Pf 2 ACAGTG L007 R2.fastqsanger)-BWA.bam
}-BWA.bam
9.9 Mb
format: bam, database: 2
Info: Etape 1
Indexation : /usr/local/bioinfo/bin/bwa index -a is
/work/galaxy/database/files/011/dataset_11741.dat >>
./bwaindex.log 2>&1

Etape 2
Alignement du premier fastq : /usr/local/bioinfo/bin/bwa
aln /work/galaxy/database/files/011/dataset_11741.d

ENSDART00000112989	2064	0	0
ENSDART00000122537	579	0	0
ENSDART00000129800	810	0	0
ENSDART00000099219	2553	0	0
ENSDART00000079354	1245	0	0
ENSDART00000024841	1405	0	0

(*) Outils Sigenae

Accès à plus d'options de l'outil

Affichage de la ligne de commande et des étapes de traitement

1

User Welcome smaman

- Logged in as smaman@toulouse.inra.fr
- Logout
- Saved Histories
- Saved Datasets**
- Public Name

2

Dataset Name	Tags	Created
{PFB-68_8.fastqsanger}-cutadapt.fastq	TESTS ***** Pierre francois mirdeep2	0 Tags 2 days ago
{PFB-69_19.fastqsanger}-cutadapt.fastq-report.html	TESTS ***** Pierre francois mirdeep2	0 Tags 2 days ago
{PFB-69_19.fastqsanger}-cutadapt.fastq	TESTS ***** Pierre francois mirdeep2	0 Tags 2 days ago
{PFB-70_20.fastqsanger}-cutadapt.fastq-report.html	TESTS ***** Pierre francois mirdeep2	0 Tags 2 days ago
{PFB-70_20.fastqsanger}-cutadapt.fastq	TESTS ***** Pierre francois mirdeep2	0 Tags 2 days ago
{PFB-71_21.fastqsanger}-cutadapt.fastq-report.html	TESTS ***** Pierre francois mirdeep2	0 Tags 2 days ago
{PFB-71_21.fastqsanger}-cutadapt.fastq	TESTS ***** Pierre francois mirdeep2	0 Tags 2 days ago
{PFB-72_22.fastqsanger}-cutadapt.fastq-report.html	TESTS ***** Pierre francois mirdeep2	0 Tags 2 days ago
{PFB-72_22.fastqsanger}-cutadapt.fastq	TESTS ***** Pierre francois mirdeep2	0 Tags 2 days ago

For 0 selected datasets: [Copy to current history](#) **Get path list**

3

1: dataset path list
2 lines, 1 comments
format: tabular, database: ?

```
#dataset_path
/work/galaxy/database/files/038/datas
/work/galaxy/database/files/038/datas
```

4

*** Save my data (version 1.0)**

dataset from your histories

Add new dataset from your history

Use file list?: Yes

Tabular file of datasets to be exported: 1: dataset path list

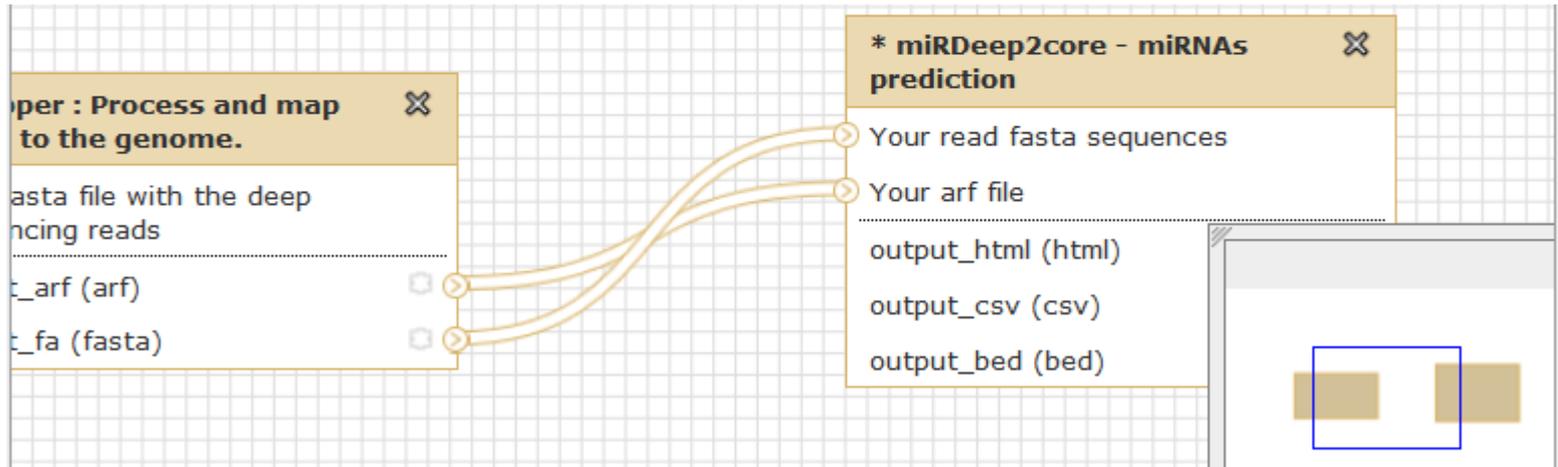
Execute

Mise en pratique prévue lors du TP.

Depuis une page blanche, vous pouvez concevoir un workflow.

Aide : les résultats produits sont typés, il n'est donc pas possible de brancher un dataset sur un mauvais tool !

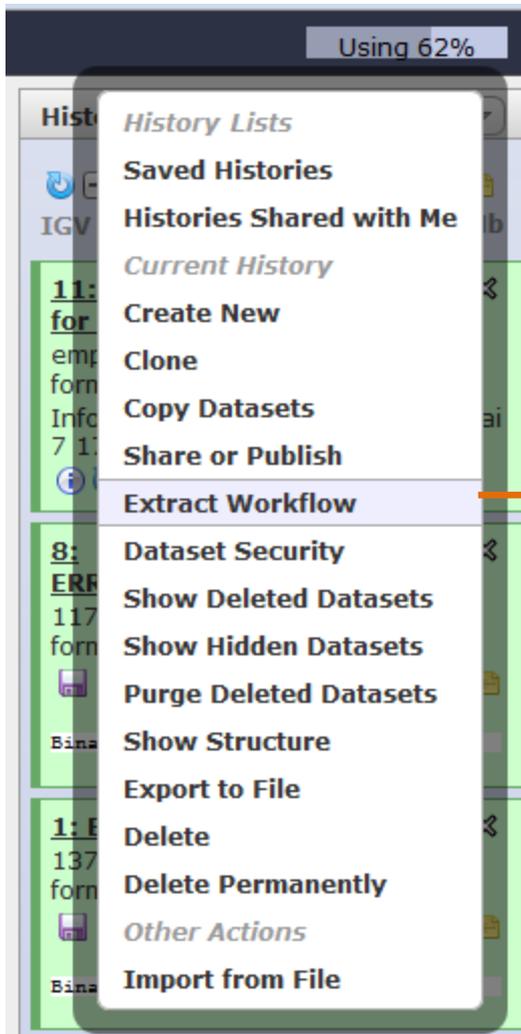




- Si tout est coché, alors tout se passe comme si rien est coché.
- Si le dataset n'est pas coché, alors qu'au moins un autre est coché, alors le dataset non coché ne sera pas visible dans l'historique. Sauf si l'utilisateur choisi d'afficher les fichiers cachés, alors, dans cette config uniquement, il pourra le voir.
-
- Si un 2ieme (ou plus) dataset est coché , alors il sera visible dans l'historique après analyse.



Depuis votre fenêtre « History », vous pouvez extraire un workflow.



Workflow name

Tool	History items created
<input checked="" type="checkbox"/> * Upload local file from filesystem path <input checked="" type="checkbox"/> Include "*" Upload local file from filesystem path" in workflow	▶ 1: ERR000017.bam
<input checked="" type="checkbox"/> * Upload local file from filesystem path <input checked="" type="checkbox"/> Include "*" Upload local file from filesystem path" in workflow	▶ 8: ERR000017.sorte
<input checked="" type="checkbox"/> * BAM sorted to BAI for IGV <input checked="" type="checkbox"/> Include "*" BAM sorted to BAI for IGV" in workflow	▶ 11: * BAM sorted to

Cliquer sur le menu « Workflow » pour lister vos workflows :

Vous pouvez ensuite, depuis le menu « Options », soit :

- Editer votre workflow pour le commenter et/ou le modifier.
- Run workflow pour lancer simultanément vos jobs.

Présentation de la plateforme Galaxy.

Premiers pas dans l'instance.

Notions d'outils, d'historique et de workflow.

Lancement de traitements bioinformatiques.

Quelques statistiques.

Auto-formations disponibles en ligne.

1 - UPLOAD YOUR DATA

Get Data

2 - FILES MANIPULATION

Text Manipulation

Filter and Sort

Join, Subtract and Group

Convert Formats

3 - SEQUENCES MANIPULATION

FASTA manipulation

FASTQ manipulation

SAM/BAM manipulation : Picard (beta)

SAM/BAM manipulation : SAM Tools

4 - MAPPING

BWA - Bowtie

5 - INDEL ET SNP

Indel Analysis

Menu évolutif et organisé par thématique

Ajout d'outils sur demande :

- Tool Shed
- Wrapper à façon (scripts maison, outils bioinfos)

-> Envoyer vos demandes à :
sigenae-support@listes.inra.fr



Ces outils sont nombreux et constituent une bonne alternative à la ligne de commande.

Voici les principaux outils « non bioinfo » proposés :

- Join (ex : fichiers lourds), Substract and Group
- Text Manipulation
- Filter and sort
- Convert Formats

Select first (version 1.0.0)

Select first:

lines

from:

What it does

This tool outputs specified number of lines from the **beginning** of a dataset

Example

Selecting 2 lines from this:

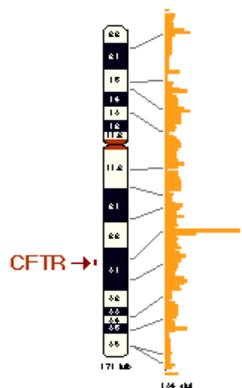
```
chr7 56632 56652 D17003_CTCF_R6 310 +
chr7 56736 56756 D17003_CTCF_R7 354 +
chr7 56761 56781 D17003_CTCF_R4 220 +
chr7 56772 56792 D17003_CTCF_R7 372 +
chr7 56775 56795 D17003_CTCF_R4 207 +
```

will produce:

```
chr7 56632 56652 D17003_CTCF_R6 310 +
chr7 56736 56756 D17003_CTCF_R7 354 +
```

Mapper un FASTQ sur une référence avec BWA.

The CFTR gene maps to chromosome 7



NGS: Mapping

- Lastz map short reads against reference sequence
- Lastz paired reads map short paired reads against reference sequence
- Map with Bowtie for Illumina
- Map with Bowtie for SOLiD
- Map with BWA for Illumina

Map with BWA for Illumina (version 0.7.17)

Will you select a reference genome?

Use one from the history ▾

Select a reference from history:

29: ERR000017_ref.fasta ▾

Is this library mate-paired?:

Single-end ▾

FASTQ file:

30: ERR003037.fastqsanger ▾

FASTQ with either Sanger-scaled quality

BWA settings to use:

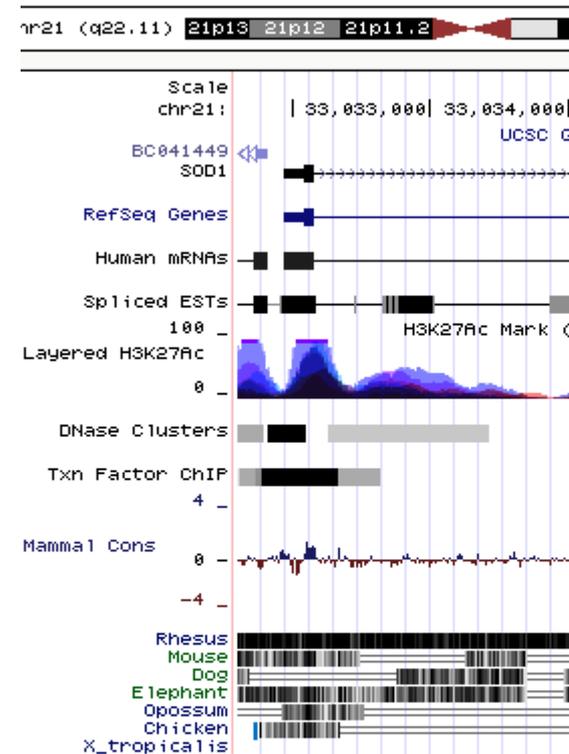
Commonly Used ▾

For most mapping needs use Commonly Used

Suppress the header in the output

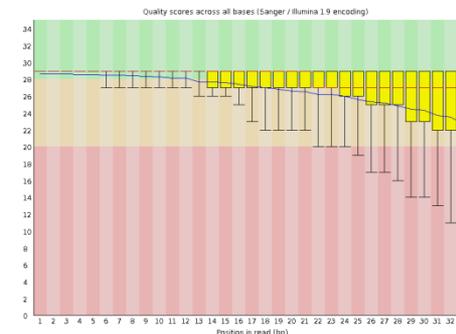
BWA produces SAM with several lines

Execute



Visualiser la qualité des données avec FASTQC Report.

Visualiser un génome avec UCSC .

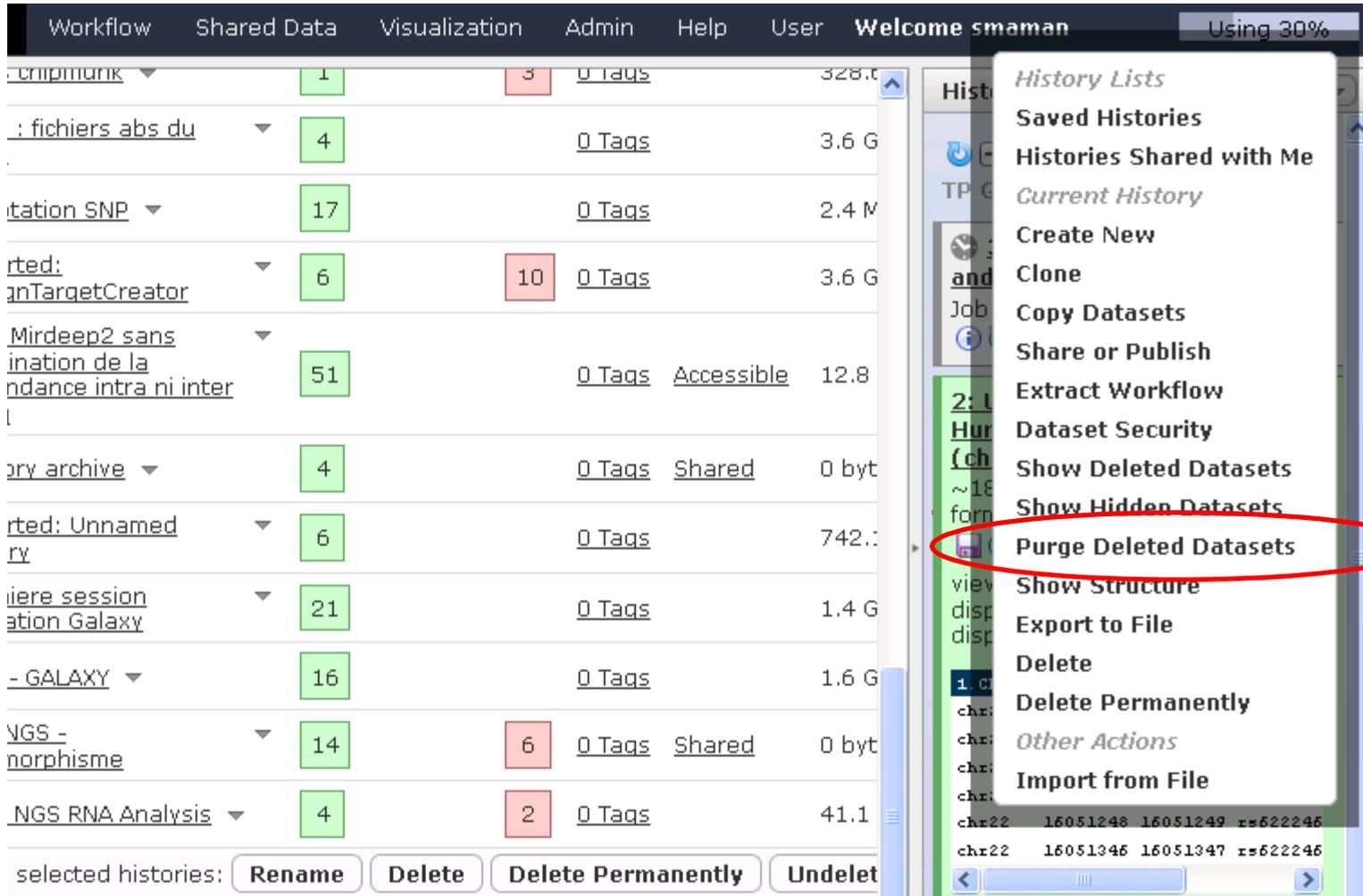


Exercice 2 & 3 :

Utilisation d'outils de traitements (bio-)informatiques.

Création et partage d'historiques et de workflows.

Suppression des datasets, historiques et workflows inutiles.
 Suppression temporaire ≠ Suppression permanente (purge)
 Organiser son espace de travail pour maîtriser son quota.



The screenshot shows the Galaxy web interface with a list of workflows and a context menu open. The workflow list includes columns for name, number of datasets, number of deleted datasets, tags, and size. The context menu is open over a workflow, and the option 'Purge Deleted Datasets' is circled in red.

Workflow Name	Number of Datasets	Number of Deleted Datasets	Tags	Size
cripmunk	1	3	0 Tags	328.0
: fichiers abs du	4	0	0 Tags	3.6 G
ation SNP	17	0	0 Tags	2.4 M
rted: anTargetCreator	6	10	0 Tags	3.6 G
Mirdeep2 sans ination de la ndance intra ni inter	51	0	0 Tags Accessible	12.8
ry_archive	4	0	0 Tags Shared	0 byt
rted: Unnamed ry	6	0	0 Tags	742.0
iere session ation Galaxy	21	0	0 Tags	1.4 G
- GALAXY	16	0	0 Tags	1.6 G
VGS - norphisme	14	6	0 Tags Shared	0 byt
NGS RNA Analysis	4	2	0 Tags	41.1

selected histories: **Rename** **Delete** **Delete Permanently** **Undelet**

Context Menu Options:

- History Lists
- Saved Histories
- Histories Shared with Me
- Current History
- Create New
- Clone
- Copy Datasets
- Share or Publish
- Extract Workflow
- Dataset Security
- Show Deleted Datasets
- Show Hidden Datasets
- Purge Deleted Datasets**
- Show Structure
- Export to File
- Delete
- Delete Permanently
- Other Actions
- Import from File

Saved Histories

[Close Advanced Search](#)

name: 

tags: 

sharing: [private](#) | [shared](#) | [accessible](#) | [published](#) | **all**

status: **active** | [deleted](#) | [all](#)

<input type="checkbox"/> <u>Name</u>	<u>Datasets</u>		<u>Tags</u>
<input type="checkbox"/> TP Galaxy project ▼	2	1	0 Tags
<input type="checkbox"/> miRNA tests ▼	59		21 0 Tags
<input type="checkbox"/> TP SNPs calling ▼	84		9 0 Tags
<input type="checkbox"/> TP RNAseq ▼	88		1 0 Tags
<input type="checkbox"/> test TP miRNA ▼	36	1	1 0 Tags

Comment citer Galaxy dans vos publications ?

- ü Les outils utilisés (nom, version).
- ü Le workflow généré.
- ü Les références Sigenae et « Galaxy project ».



How to cite Galaxy workbench ?

Depending on the help provided you can cite us in acknowledgements, references or both.

Examples :

Acknowledgements

We wish to thank the SIGENAE group for

References

X. SIGENAE [<http://www.sigenae.org/>]

Primary Publications

If you use or extend Galaxy in your published work, please cite **each** of the following publications:

1. Goecks, J, Nekrutenko, A, Taylor, J and The Galaxy Team. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol.* 2010 Aug 25;11(8):R86.
2. Blankenberg D, Von Kuster G, Coraor N, Ananda G, Lazarus R, Mangan M, Nekrutenko A, Taylor J. "Galaxy: a web-based genome analysis tool for experimentalists". *Current Protocols in Molecular Biology.* 2010 Jan; Chapter 19:Unit 19.10.1-21.
3. Giardine B, Riemer C, Hardison RC, Burhans R, Elnitski L, Shah P, Zhang Y, Blankenberg D, Albert I, "Galaxy: a platform for interactive large-scale genome analysis." *Genome Research.* 2005 Oct; 15(:

Présentation de la plateforme Galaxy.

Premiers pas dans l'instance.

Notions d'outils, d'historique et de workflow.

Lancement de traitements bioinformatiques.

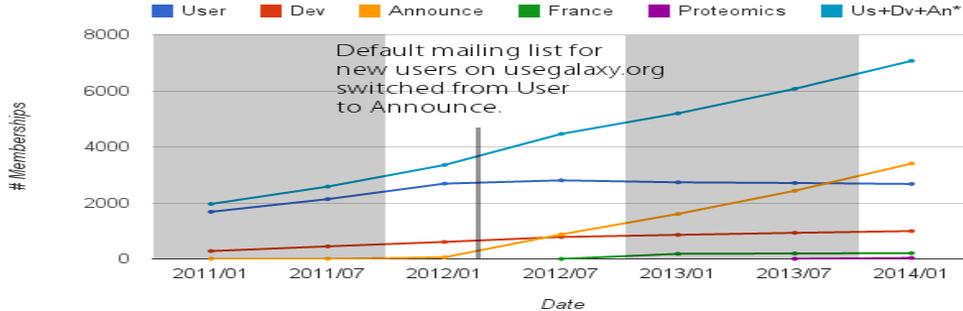
Quelques statistiques.

Auto-formations disponibles en ligne.

Statistiques du Galaxy Project

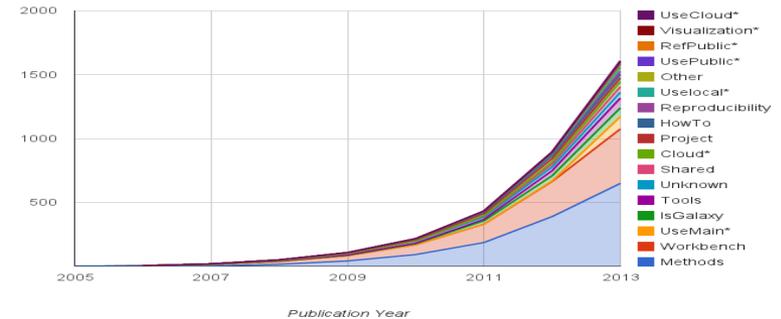
<https://wiki.galaxyproject.org>

Galaxy Mailing List Memberships



Une communauté internationale vivante.
Une communauté française grandissante.

Publications: Cumulative Tags per Year 2005-2013*



Belle augmentation des citations Galaxy dans les publications

Statistiques de l'instance Galaxy Sigeneae / BioInfo Genotoul

<http://galaxy-workbench.toulouse.inra.fr>

Historique mensuel des visites



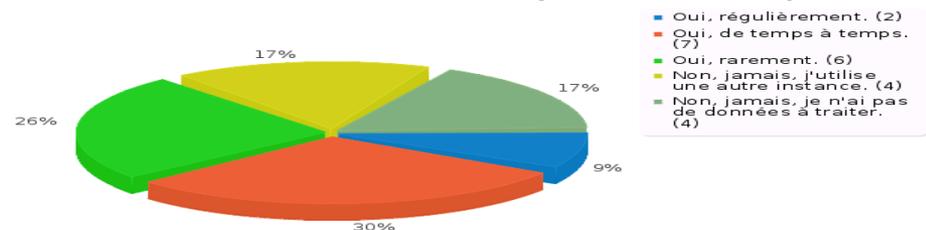
23 réponses sur ~40 utilisateurs

~40 utilisateurs

Les 10 plus gros utilisateurs (hors tests Sigeneae) utilisent Galaxy dans le cadre de leur projet.

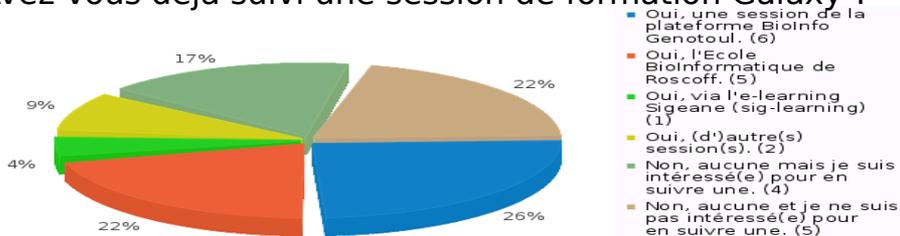
65% des utilisateurs Galaxy ayant répondu à l'enquête utilisent l'instance Sigeneae

Utilisez-vous l'instance Sigeneae de Galaxy ?



Importance de la formation et du e-learning

Avez-vous déjà suivi une session de formation Galaxy ?



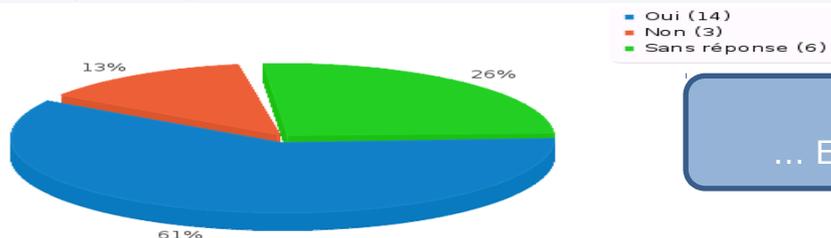
6% des utilisateurs seulement ont déjà suivi une formation en e-learning.

60% des utilisateurs :

- ont déjà suivi une formation Galaxy -> Besoin d'une 'mise en route',
- souhaitent plus de support en bioinfo.
- créent et utilisent des workflows

Les traitements Galaxy sont aussi lancés dans le cadre de projets

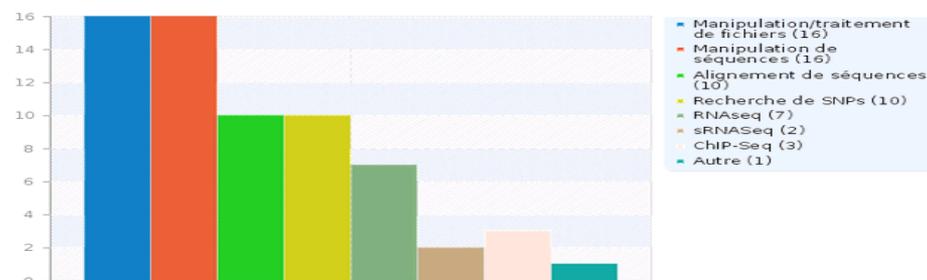
Galaxy vous permet-il de traiter vos données bio-informatiques ?



~60% traitent leurs données dans Galaxy.
... Et 60% de ces 60% utilisent ces données dans leur projet.

Principalement des applications de type SNP et RNAseq

Quels sont les principaux outils utilisés ?



Présentation de la plateforme Galaxy.

Premiers pas dans l'instance.

Notions d'outils, d'historique et de workflow.

Lancement de traitements bioinformatiques.

Quelques statistiques.

Auto-formations disponibles en ligne.

Une FAQ et le lien vers « sig-learning » sont disponibles depuis la page d'accueil

Shared Data Lab Visualization Admin Help User User **Welcome smaman,**



FAQ on your Galaxy tool

▼ Dataset, history and workflow ?

Step 1 : Import your datasets

First of all, you have to import your data files thanks to "Data Analysis / Get Data" tool. Then your downloaded datasets are automatically archived in "User / Saved Datasets".

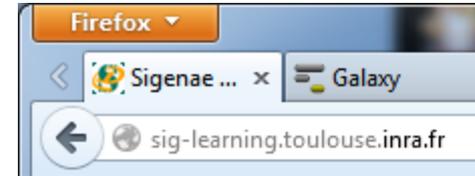
Step 2 : Select tools and create your history

Then you select relevant tool in "Data Analysis", on the left side of Galaxy interface.

Vos supports sont disponibles depuis : **<http://sig-learning.toulouse.inra.fr>**

"If you need more training about bioinformatic and Galaxy, please connect to [Sigenae e-learning platform](http://sig-learning.toulouse.inra.fr)."

- 1 Taper l'adresse de « sig-learning » :
http://sig-learning.toulouse.inra.fr/
Ou directement depuis Galaxy



Sigenae e-learning platform

If you need more training about bioinformatic and Galaxy, please connect to [Sigenae e-learning platform](#)

Some of the tools have a direct access to the e-learning platform of sigenae. Those tools will have this  in the help section. Click on this icon to be redirected to the e-learning platform.

Sigenae support : sigenae-support@listes.inra.fr

If you have some question about Galaxy, please consult your [FAQ](#)

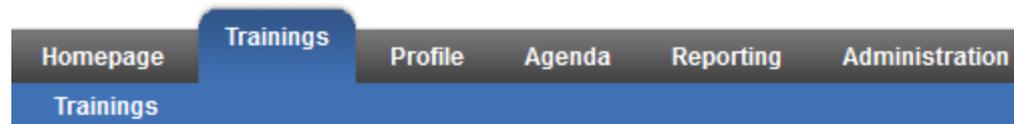
- 2 Authentification

Login

Pass

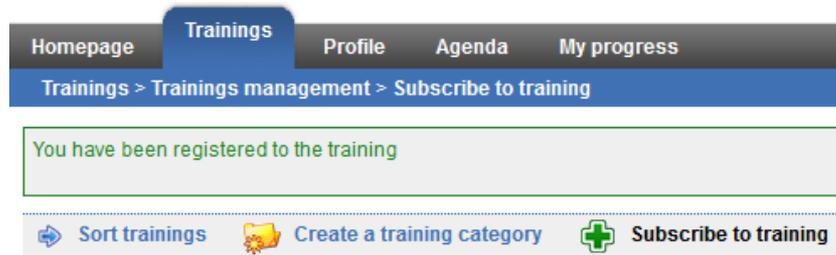
 Enter

- 3 Onglet « Trainings » pour accéder à vos e-formations :

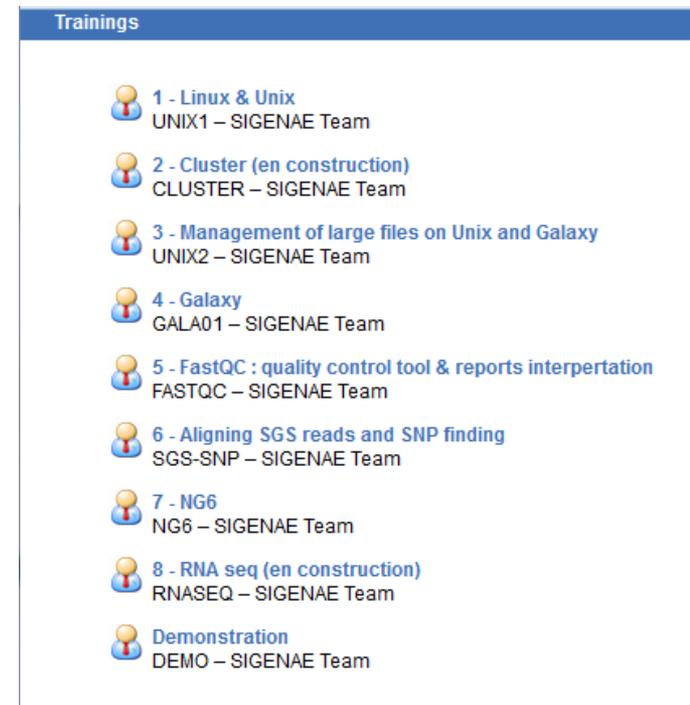


-  **1 - Linux & Unix**
UNIX1 – SIGENAE Team
-  **2 - Cluster (en construction)**
CLUSTER – SIGENAE Team
-  **3 - Management of large files on Unix and Galaxy**
UNIX2 – SIGENAE Team

Il vous est possible de vous inscrire directement en ligne à une formation :
« Trainings » « Trainings management » puis « Subscribe to training » :



L'inscription s'effectue via une recherche de la formation par mots clés.
Voici donc la liste des formations :



Training home

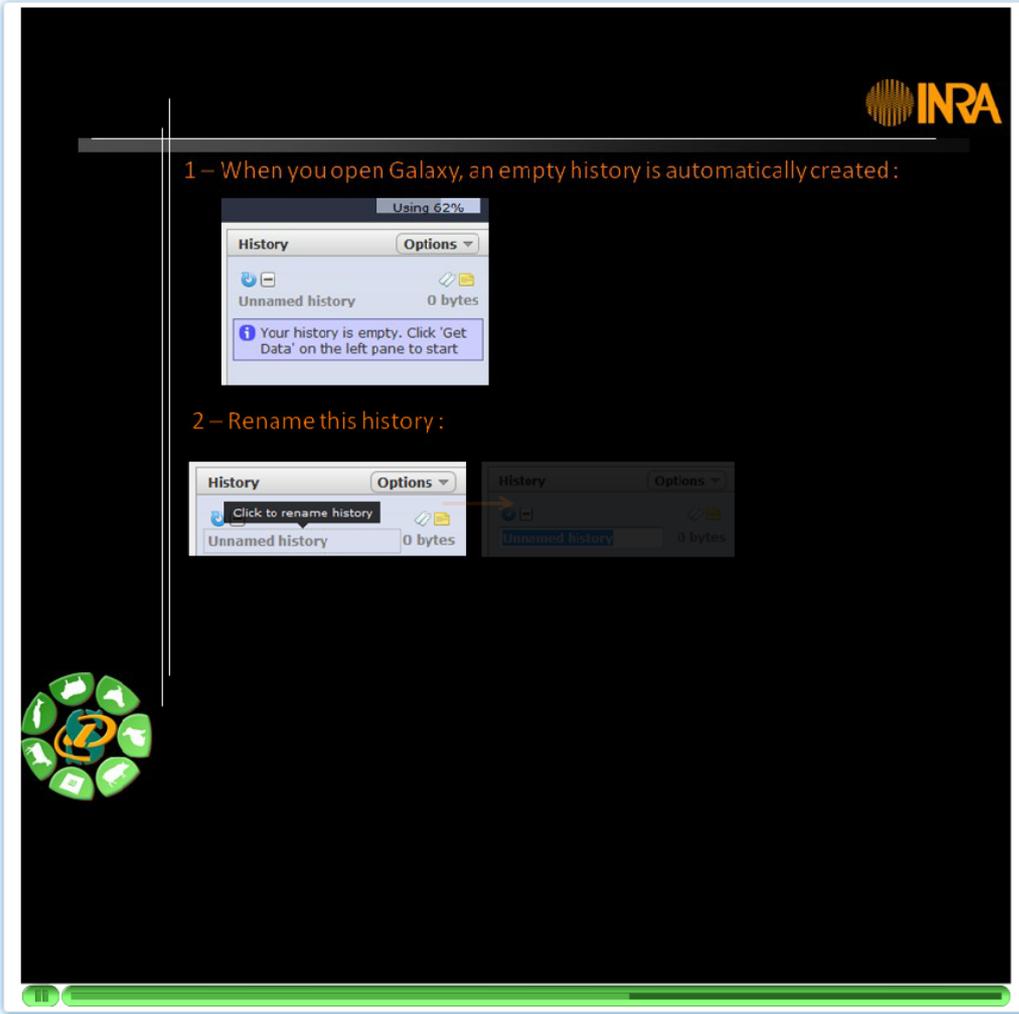
Galaxy 57%

Galaxy > Galaxy User Interface

1 - Galaxy User Interface

Build Organize Display

- Galaxy menu
 - Galaxy presentation ✓
 - Galaxy connexion ✓
 - Your screen is divided in 3 parts ✓
 - Menu ✓
- Import Dataset
 - How to get data ? ✓
 - Upload without copy on server ✓
 - Upload with copy on server ✓
 - Dataset from NG6 ✓
 - UCSC ✓
 - NCBI ✓
 - BIOMART: Extract tab files ✓
- Datasets, tools and history**
 - History creation ✓**
 - Manage histories
 - Share histories
 - Datasets and tools are listed in your history
- Edit, run and share a workflow
 - Create a workflow
 - Edit and run a workflow
 - Share workflows
 - Import your workflow
 - Export workflow
 - Main steps



1 – When you open Galaxy, an empty history is automatically created :

2 – Rename this history :

Commentaires Comments

Lorsque vous vous connectez à Galaxy, un historique vide est automatiquement créé de gauche.

Tout d'abord, il est recommandé de renommer votre historique et de télécharger vos jeux de données avec les outils disponibles dans « G »

Merci pour votre écoute

Questionnaire
<http://bioinfo.genotoul.fr/index.php?id=79>



25: demo.fasta

Job is currently running

24:

http://genoweb.toulouse.inra.fr/~sigenae/sarah/Galaxy_Formation/test-data/1.fasta

1 sequences
format: fasta, database: ?
uploaded fasta file

```
>hg17
gtttgccatcttttgcctcttaggaaatccagcagctgtca
CTATAAAGACCACCTTTTAAAACTTCCC-----
tgtgtgataatggtcttcagttaca-cagaaattcttctt
atttaaaaaaaaaactttgagc-----tagacaccaggctatg
GTTTCCATTAGGAAGCCTCGAATGCAATGTGACTGTGCTC
```

Sigenae - Welcome smaman-1 Workflow Shared Data Visualization Admin Help User Using 164

Tools

search tools

UPLOAD YOUR DATA

Get Data

- Upload File
 - * Upload File from genotoul
 - * **EBI SRA** ENA SRA
 - UCSC Main table browser
 - UCSC Test table browser
 - UCSC Archaea table browser
- Get Microbial Data
 - BioMart Central server
 - * Upload my data to work on Genotoul
 - * Save my data on Genotoul

FILES MANIPULATION

- Text Manipulation (e-learning)
- Filter and Sort
- Join, Subtract and Group
- Convert Formats
- BED Tools
- Graph/Display Data

SEQUENCES MANIPULATION

- FASTA manipulation
- FASTQ manipulation (e-learning)
- SAM/BAM manipulation : Picard (beta)
- SAM/BAM manipulation: SAMtools (e-learning)

```
>EYKX4VC01B65GS length=54 xy=0784_1754 region=1 run=R_2007_11_07_16_15_57_
CCGGTATCCGGGTGCCGTGATGAGGCCACCGGAACGAATTCGACTATGCCGAA
>EYKX4VC01BNCSP length=187 xy=0558_3831 region=1 run=R_2007_11_07_16_15_57_
CTTACCGGTCACCACCGTGCCTTCAGGATTGATCGCCAGATCGGTGCGTCAAGGCGG
GGTGACATCGCCACCACCGTACTCACTGGCTGGCTCTGGTCCCGCGGCCATCGGAGGC
CACCACGTTGAGGGTATCCCTCGGTTGTGGCTCGGTGAGAACCACGTTGTAGTCGCC
ATTGTC
```

History

TESTS SARAH

81.8 KB

25:

http://genoweb.toulouse.inra.fr/~sigenae/sarah/Galaxy_Formation/test-data/4.fasta

2 sequences
format: fasta, database: ?
uploaded fasta file

```
>EY04VC01B65GS length=54 xy=0784_1754
CCGGTATCCGGGTGCCGTGATGAGGCCACCGGAACGAATTCGACTATGCCGAA
>EY04VC01BNCSP length=187 xy=0558_3831
CTTACCGGTCACCACCGTGCCTTCAGGATTGATCGCCAGATCGGTGCGTCAAGGCGG
GGTGACATCGCCACCACCGTACTCACTGGCTGGCTCTGGTCCCGCGGCCATCGGAGGC
CACCACGTTGAGGGTATCCCTCGGTTGTGGCTCGGTGAGAACCACGTTGTAGTCGCC
CACCACGTTGAGGGTATCCCTCGGTTGTGGCTCGGTGAGAACCACGTTGTAGTCGCC
```

24:

http://genoweb.toulouse.inra.fr/~sigenae/sarah/Galaxy_Formation/test-data/1.fasta

1 sequences
format: fasta, database: ?
uploaded fasta file

```
>hg17
gtttgccatcttttgcctcttaggaaatccagcagctgtca
CTATAAAGACCACCTTTTAAAACTTCCC-----
tgtgtgataatggtcttcagttaca-cagaaattcttctt
atttaaaaaaaaaactttgagc-----tagacaccaggctatg
GTTTCCATTAGGAAGCCTCGAATGCAATGTGACTGTGCTC
```