GALAXY BIOINFORMATICS WORKFLOW ENVIRONMENT



Rutger Vos, 3 April 2012

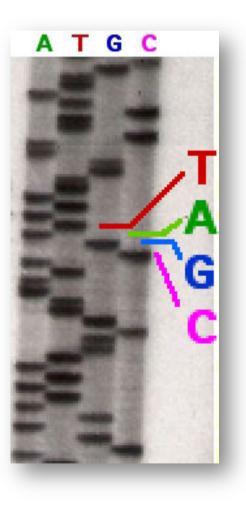
NCB naturalis

Overview

Informatics in the post-genomic era

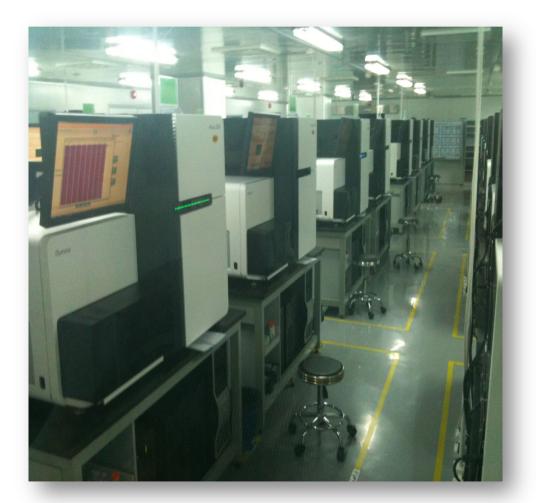
The past (?)

- Analyses glued together using scripting languages, directly on the CLI or in GUI
- □ Sanger sequencing
- Smaller data volumes
- □ Fewer remote data resources
- □ Hypothesis-driven



The present

- Graphical or textbased workflow tools
- "Next generation" sequencing
- □ Large data sets
- Many remote data resources
- □ "Data-driven"



NGS – Roche 454 pyrosequencing

Pyrosequencing

- □ "Emulsion PCR"
- Bead with primer in each droplet
- Each bead is placed in a well with luciferase
- Plate is analyzed by fiberoptic chip

Genome Sequencer FLX



NGS – Illumina/Solexa

Reversible dye-terminator seq

DNA attaches to primer on slide and is amplified

- □ 4 RT-bases are added
- Camera detects labeled nucleotides
- □ Next 1-base cycle

HiSeq2000 (BGI)



NGS – IonTorrent

Ion semiconductor sequencing

- □ "sequencing by synthesis"
- Not light-based, sensor detects H+ ions during synthesis
- □ Longer reads

Ion Torrent PGM

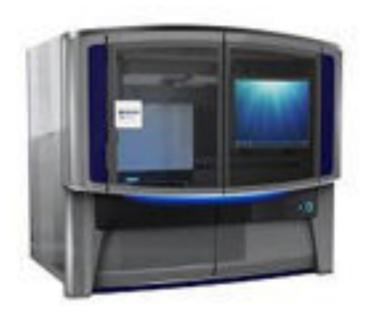


NGS – SOLiD Sequencing

Oligonucleotide ligation and detection

- Beads with DNA fragments
- Universal adapter attached to fragments
- PCR product attaches to slide
- Fluorescent probes ligate to the primer

SOLiD 5500 Genetic Analyzer



The future

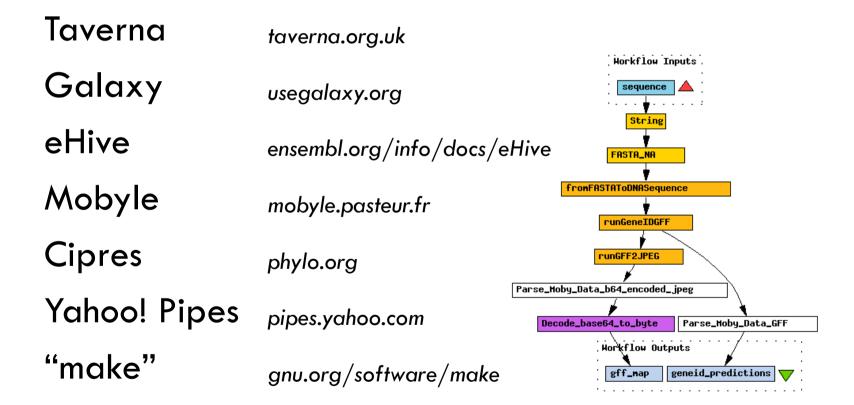
- "Next-next generation"
 sequencing (MinION?)
- □ Smaller data sets?
- □ Semantic web?
- □ Back to hypotheses?



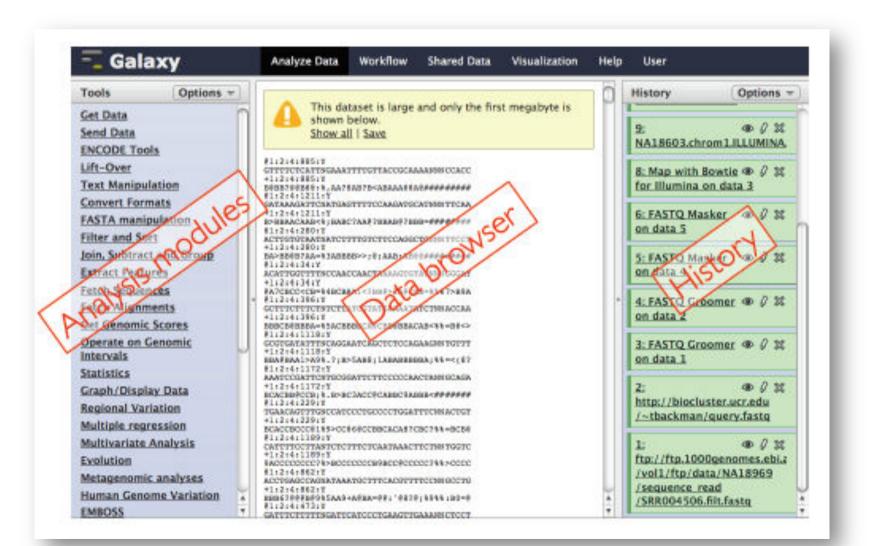


Tools for automating bioinformatics analyses

Examples of workflow tools



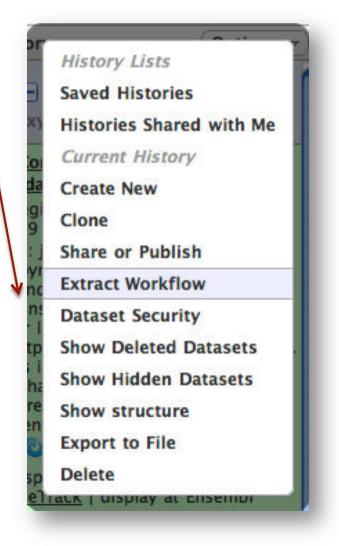
Galaxy



Provenance, histories

- Where do the data come from?
- How were they altered?
- Galaxy tracks the history of data.
- Histories can be converted to workflows

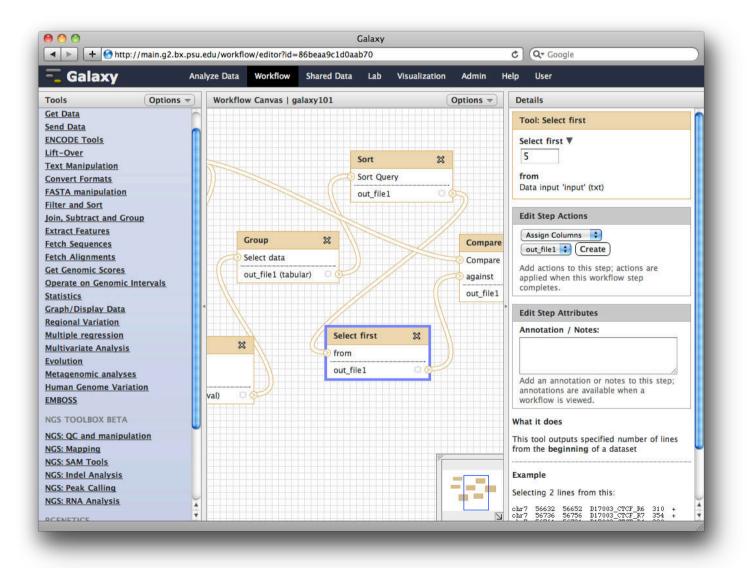
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Creating a workflow

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Workflow editor



Reproducibility

Good science requires that results be reproducible

□ Some analyses are run many times

Your workflows Create new workflow Upload or impo Name # of Steps imported: RNAseq, Part 3 = 2 imported: RNAseq, Part 2 = 3	nport workflow
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imported: RNAseq Part 2 -	
imported: RNAseq, Part 1 👻 4	
imported: mRNA SNPs example 👻 8	
imported: miRNA Secondary Analysis 👻 3	

Sharing

- "Standing on the shoulders of giants"
- Not re-inventing the wheel
- "Executable
 papers" (doi:
 10.1101/gr.
 094508.109)





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Windshield splatter analysis with the Galaxy metagenomic pipeline

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+ Author Affiliations

+ Author Notes

 \rightarrow ⁶ These authors contributed equally to this work.

Abstract

How many species inhabit our immediate surroundings? A straightforward collection technique suitable for answering this question is known to anyone who has ever driven a car at highway speeds. The windshield of a moving vehicle is subjected to numerous insect strikes and can be used as a collection device for representative sampling. Unfortunately the analysis of biological material collected in that manner, as with most metagenomic studies, proves to be rather demanding due to the large number of required tools and considerable computational infrastructure. In this study, we use organic matter collected by a

Data

What data types, expressed in which formats, does Galaxy operate on? How do I get data into and out of Galaxy?

Data types

- Sequences
- Alignments
- Intervals
- Tabular data

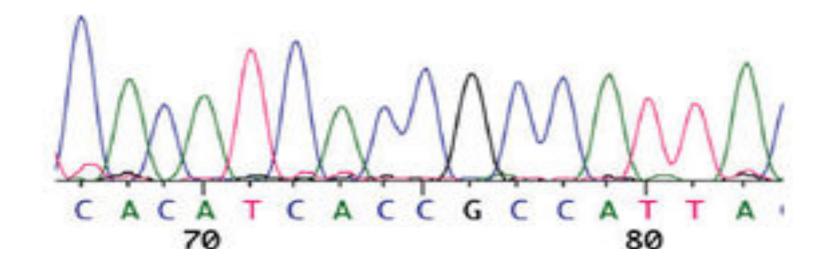
File format conversion

- Built-in converters
 between related
 file formats are
 provided
- Additional converters can be added

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 <u>BEAM</u> significant single- and multi-locus SNP associations in case-control studies 	III													1
 <u>LPS</u> LASSO-Patternsearch algorithm 														
 <u>HVIS</u> visualization of genomic data with the Hilbert curve 														
 <u>Call SNPS with Freebayes</u> Bayesian genetic variant detector 														
 <u>Convert CG MasterVar</u> file to pgSnp format 														

Galaxy sequence formats

- □ FASTA def line, sequence
- □ **FASTQ** *FASTA* + quality SOLEXA:
- □ ABI/SCF binary sequence trace (see below)
- □ SFF (454) binary flowgram format



Galaxy alignment formats

- □ **MAF** multiple alignment format (see below)
- □ (S B)AM text or binary format for reads and ref
- □ **AXT** pairwise alignment, from LAV
- □ **LAV** BLASTZ output, pairwise alignment

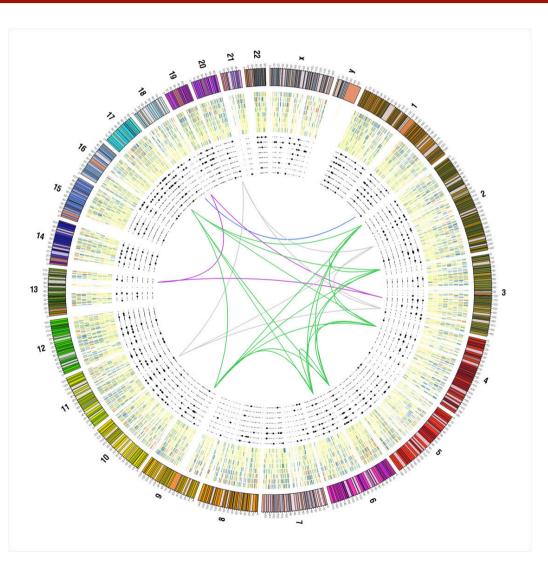
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Galaxy interval/feature formats

- □ chrom, start, end, ...
- \Box INTERVAL
 - **BED** with headers
- □ GFF (GFF3)
 - like BED and interval, but 1-based inclusive

□ WIG(GLE)

 dense, continuousvalued tracks



Other data formats

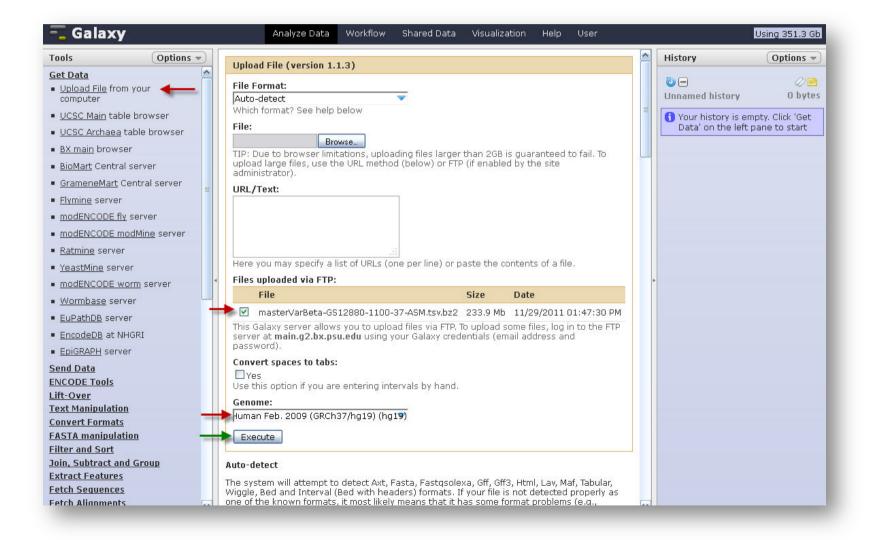
In addition to interval/feature tabular data as listed previously, other files with similar properties can be processed by some tools:

- *.txt tab-separated values (e.g. tabular FASTA)
- HTML (for additional prose)
- LPED/PBED (to describe SNPs, really two files, one for coordinates, other for alleles)

Data I/O

- Upload via FTP
- Fetch by URL
- Import from data library
- Provided by interoperable web service

Upload data using FTP



Fetch data from a URL

Galaxy	Info: <u>report bugs</u> <u>wiki</u> <u>screencasts</u> <u>blog</u>	Logg	ed in as rch8@psu.edu: r	<u>manage logout</u>
Tools	Upload File		History	Options
	Upload File File: Choose File no file selected URL/Text: http://www.bx.psu.edu/~ross/share/DukeDNas eSitesHg18.bed.txt Here you may specify a list of URLs (one per line) or paste the contents of a file. Convert spaces to tabs: Ýes Use this option if you are entering intervals by hand. File Format: Auto-detect ; Which format? See help below Genome: Human Mar. 2006 (hg18) Execute			Options RMs, @ () % @ () % § @ () %
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Import from data library

We will be performing various network	changes over t	he next few d	avs that may cause	brief downtime. I	f vou expe	rience problems for an extended	amount of t
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Bushman	Data for	Nature Lette	r "Complete Kho	isan and Bantu g	enomes fr	rom southern Africa"	
ChIP-Seg Mouse Example	Data use	ed in example	es that demonstr	ate analysis of Cl	nIP-Seq d	ata	
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Codon Usage Frequencies							
Coleman	IonPGM						
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Evolutionary Trajectories in a Phage	Experim	ental evolution	on (Illumina)				
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Fetch data from proxy service

- User submits proxy request to Galaxy
- □ Galaxy forwards request to remote service
- Service returns data
- □ Galaxy infers data type and presents results



"Big Data"

- NGS has led to massive data sets
- Data formats are simple, binary, and/or compressed
- Still, people drive around with USB hard disks



Data sharing/publishing

The Galaxy platform

allows users to publish and share their data, for example as supplemental materials to a publication*

Name	Message	Data type	Date uploaded	File siz
Trip A Left Side Reads v		fasta	2009-08-21	20.4 Mt
Trip A Left Side QV -		qual454	2009-08-21	49.8 Mt
Trip A Right Side Reads -		fasta	2009-08-21	31.6 Mb
Trip A Right Side QV -		qual454	2009-08-21	76.7 Mb
Trip B Left Side Reads		fasta	2009-08-21	24.0 Mb
Trip B Left Side QV -		qual454	2009-08-21	57.5 Mb
Trip B Right Side Reads		fasta	2009-08-21	22.7 Mb
Trip B Right Side QV -		qual454	2009-08-21	53.3 Mb
For selected datasets: Import	to current history	; (Go)		
TIP: You can download individual			wnload this dataset"	from the
context menu (triangle) next to ea	ach dataset's name	2 CO		

bzip2: Recommended for slower network connections (smaller size but takes longer to compress)

* example: http://genome.cshlp.org/content/19/11/2144

decompresses it on the fly.

Tools

Which operations can I run on Galaxy?

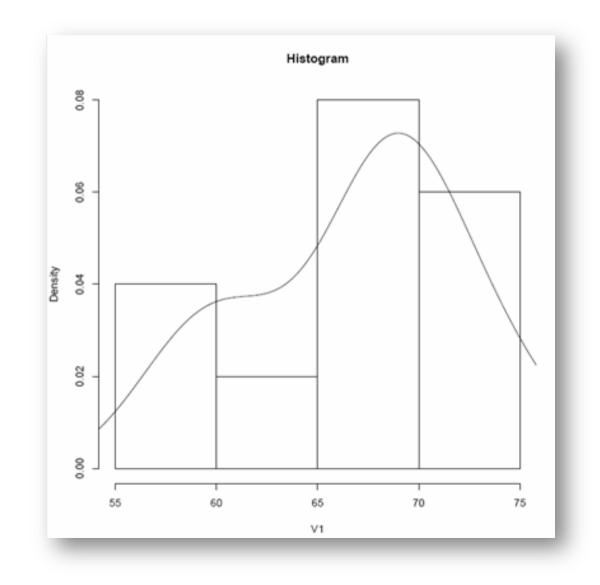
Galaxy tools

- □ Send and Get data Upload, fetch, send, submit
- Data manipulation Join, sort, filter
- □ Format conversion FASTA, other format operations
- □ Statistics Regressions, simulations, model tests
- □ NGS BAM, FASTQ, SOLiD, 454 file operations
- RNA analysis cufflinks, tophat
- Evolution branch lengths, NJ, HyPhy

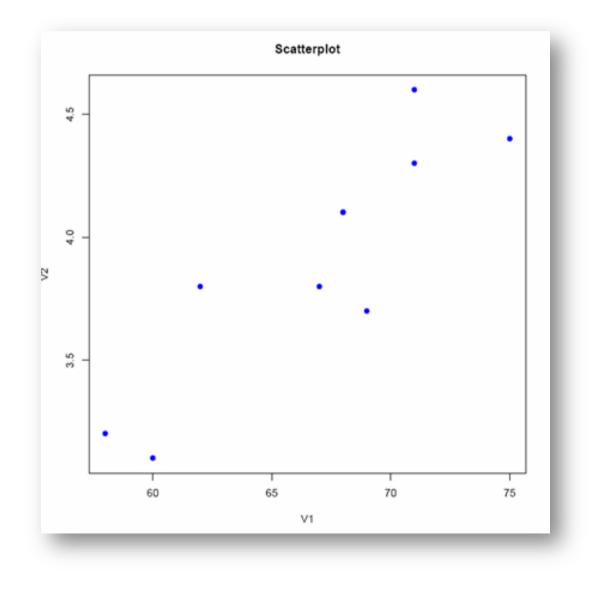
Visualization

What data can I view in Galaxy?

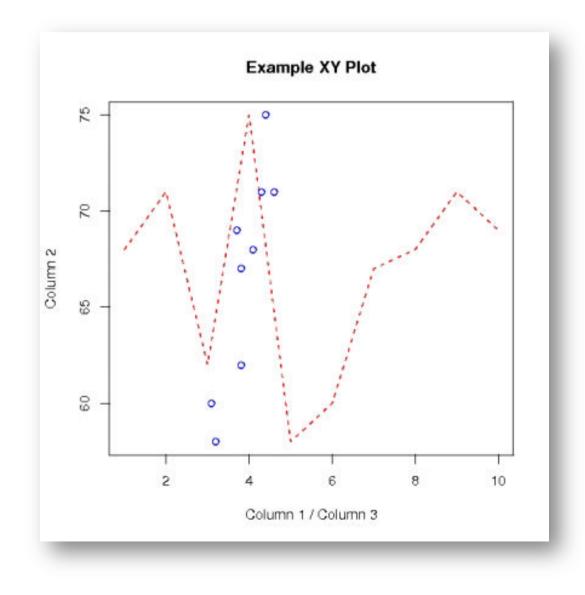
Galaxy visualization - histograms



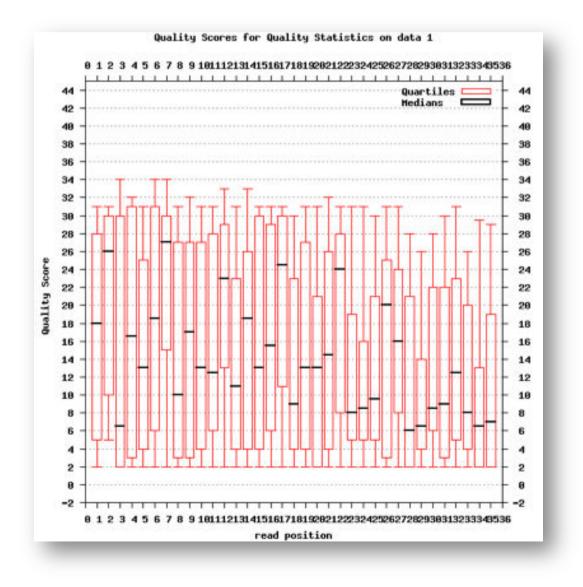
Galaxy visualization - scatterplots



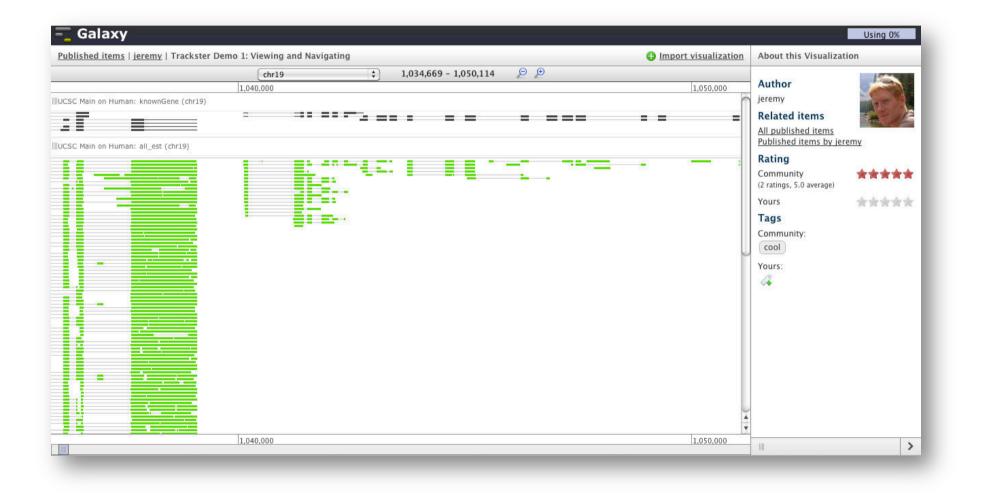
Galaxy visualization – XY plot



Galaxy visualization – box plot



Galaxy visualization - trackster



Deployment

How to deploy your analyses on Galaxy, on public servers, in the cloud or locally

Public servers

- "Main" at UseGalaxy.org
- NBIC
- Others listed on Galaxy wiki

Local server

Pro:

Con:

- Data close by
- □ Can add your own tools
- Can develop Galaxy further
- UNIX-based

- □ Complicated install
- □ Many dependencies
- UNIX-based

Cloud Galaxy

□ Galaxy can be installed in the (Amazon EC2 cloud)

Private data without the hardware hassle

Uploading and storing data can be costly, however

Galaxy	CloudMan C	onsole		
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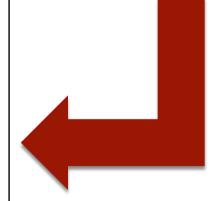
Implementation

How does it work under the hood?

Galaxy under the hood

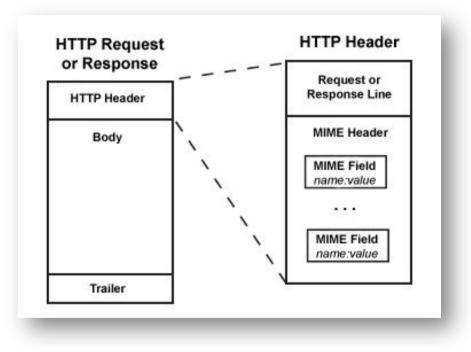


- 1. Parses HTTP request
- 2. Identifies which tool to use
- 3. Reads tool description
- 4. Queues tool
- 5. Parses result
- 6. Returns HTML representation of result



Web server

- Simple Galaxy installs use a built-in, python-based HTTP server
- More robust installs
 typically use the Apache
 httpd server



Code base

- Most of the framework and the wrapper code is written in python
- Some wrappers in other languages, e.g. perl

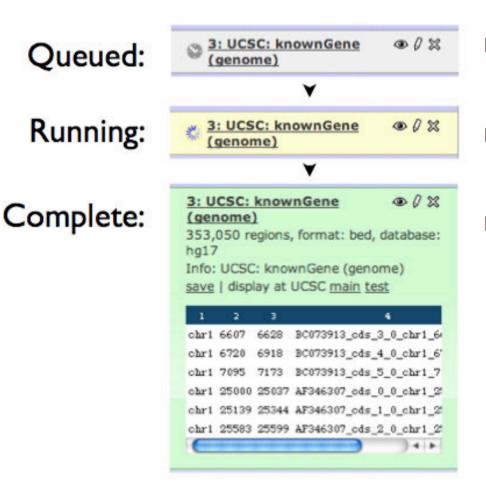


Interface language

- Under the hood, Galaxy executes command-line programs and scripts
- □ Their interfaces and tool tips are described in XML files



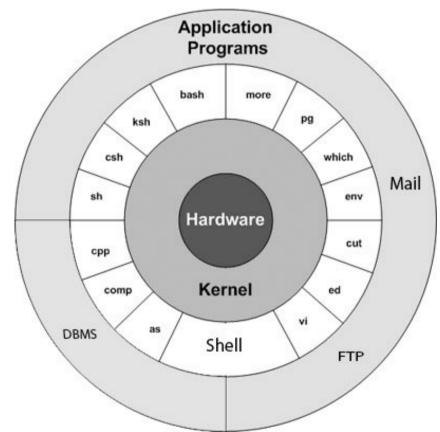
Queuing



- Jobs are executed asynchronously
- Progress is shown in the data browser
- On big servers (e.g. "Main"), queuing is managed by the dedicated "Torque" system

UNIX

- Galaxy (simply)executes command-line programs within a UNIX-like environment
- Galaxy doesn't have to "know" how to run those programs, it finds out from their descriptions at runtime



Database

- Analysis metadata is stored
 in a database, by default
 this is SQLite
- More robust installs use
 PostgreSQL



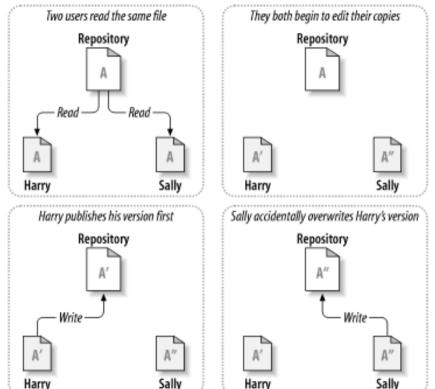
Configuration

- Galaxy has many moving parts that can be configured
- Configuration is done using simple text-based INI files



Version control

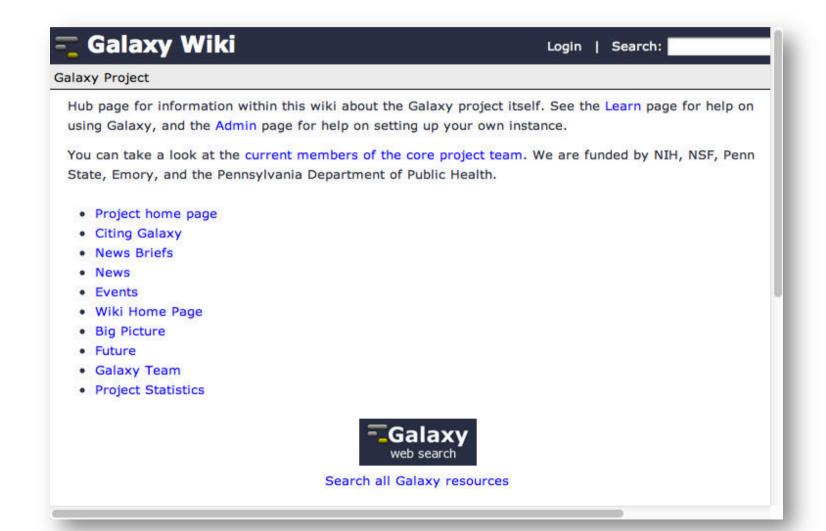
- Revision control (or version control) provides unlimited undo and detailed tracking of changes
- Galaxy uses Mercurial
- Popular now are svn, git and hg



Community

How to get in touch with the world-wide community to get the most out of Galaxy?

Wiki



Mailing lists

- □ @lists.bx.psu.edu:
 - galaxy-user
 - galaxy-dev
 - galaxy-announce
 - galaxy-commits



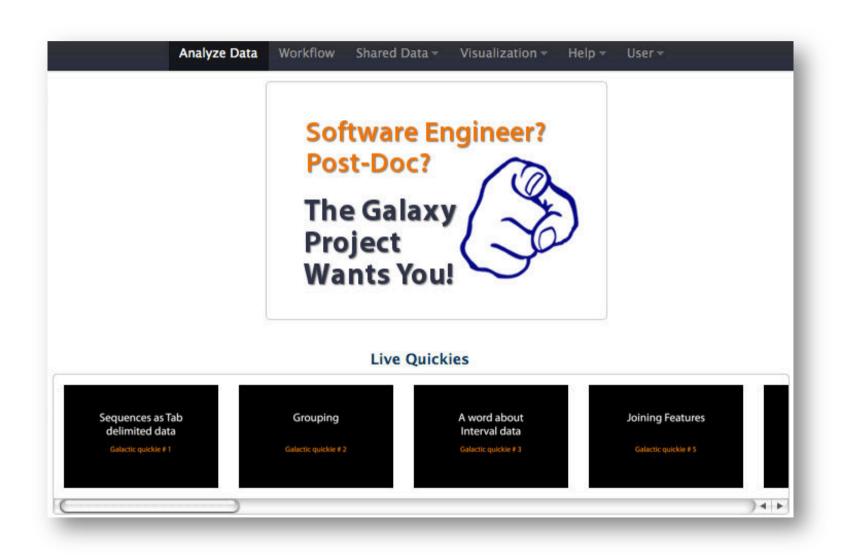
Tutorials

Galaxy 101:

- Getting data from UCSC
- Performing simple data manipulation
- Understanding Galaxy's History system
- Creating and editing workflows
- Applying workflows to your data



Screencasts



Events

- Galaxy Community
 Conference
- \square ISMB
- □ ECCB
- D PAG
- Bio-IT World
- □ GMOD Meetings





"Tool shed"

1234 valid tools on Apr 02, 2012	Categories			
iearch Search for valid tools	search repository name, description			
Search for workflows	Name	Description	Repositories	
Repositories	Assembly	Tools for working with assemblies	14	
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Login to create a repository	Convert Formats	Tools for converting data formats	21	
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	SAM	Tools for manipulating alignments in the SAM format	14	
	Sequence Analysis	Tools for performing Protein and DNA/RNA analysis	86	
	SNP Analysis	Tools for single nucleotide polymorphism data such as WGA	9	
	Statistics	Tools for generating statistics	18	
	Text Manipulation	Tools for manipulating data	21	
	Visualization	Tools for visualizing data	21	
	Web Services	Tools enabling access to web services	1	

- Easy sharing of new tools
- Based on Mercurial
- Turns Galaxy into a modular ecosystem



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Social citation manager

□ There is a Galaxy group:

□ citeulike.org/group/16008

Articles are tagged by:

PROJECT, ISGALAXY, SHARED, HOWTO, METHODS, REPRODUCIBILITY, WORKFLOW

Organizations

- Development:
 - Penn State University
 - Emory University
- □ Support:
 - NSF
 - NHGRI
- Power user:NBIC





Links

- □ UseGalaxy.org the Main server
- □ GetGalaxy.org for local installs
- □ UseGalaxy.org/galaxy101 intro tutorial
- □ Galaxy.nbic.nl Sombrero, the NBIC server
- □ CiteULike.org/group/16008 references
- □ genome.cshlp.org/content/19/11/2144 windshield paper
- SlideShare.net/rvosa these slides