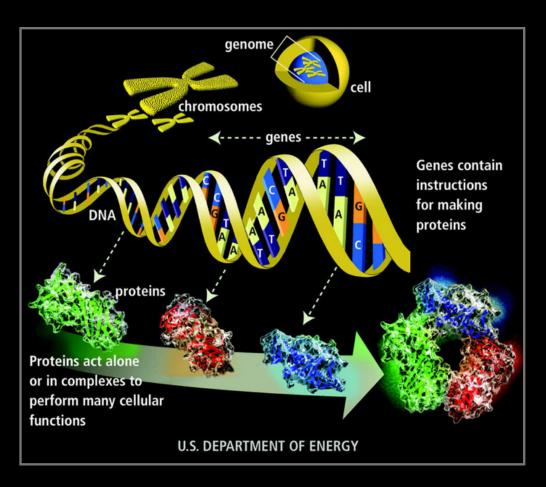
Galaxy: A Web-based Platform for Accessible, Reproducible, and Transparent High-throughput (Genome) Biology

Jeremy Goecks
Depts. of Biology and Math & Computer Science
Emory University

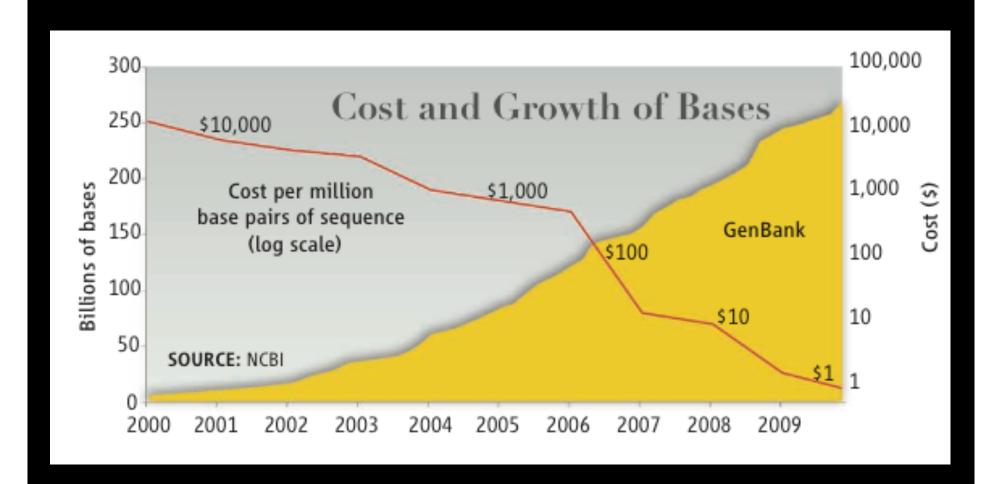
Genomics



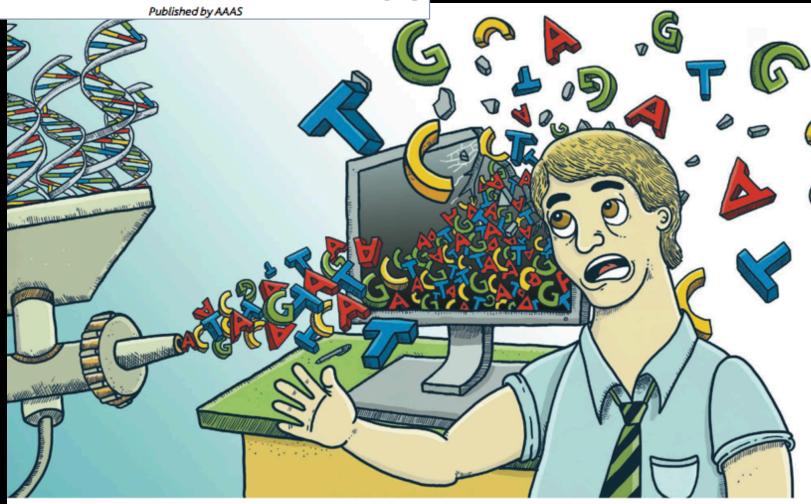
Identify and annotate all functional genomic elements

Understand interactions among elements

Apply genome knowledge to address biomedical challenges



11 FEBRUARY 2011 VOL 331 SCIENCE www.sciencemag.org



Will Computers Crash Genomics?

New technologies are making sequencing DNA easier and cheaper than ever, but the ability to analyze and store all that data is lagging

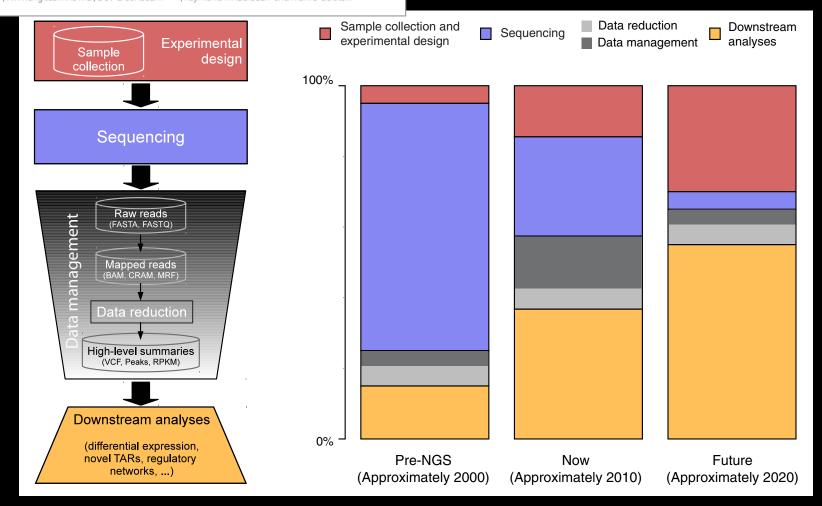
you-go service, accessible from one's own desktop, that provides rented time on a large cluster of machines that work together in parallel as fast as, or faster than, a single powerful computer. "Surviving the data deluge means computing in parallel," says Michael www.sciencemag.org on February 10, 2011



OPINION

The real cost of sequencing: higher than you think!

Andrea Sboner^{1,2}, Xinmeng Jasmine Mu¹, Dov Greenbaum^{1,2,3,4,5}, Raymond K Auerbach¹ and Mark B Gerstein*^{1,2,6}



A Key Challenge in Genomics

Generating data is easy

- high-throughput/next-generation sequencing (HTS/NGS) technologies improving rapidly
- datasets are many MBs or GBs

Analyzing data is THE bottleneck

computation is essential due to dataset size

Computation in Science?

Scientists unfamiliar with computation

Reproducibility hindered by complexity: systems, scripts, tools, parameters

Collaboration and publishing difficult because current media do not support computational artifacts well

Galaxy Project: Fundamental Questions

When Biology (or any science) becomes dependent on computational methods:

- how best to make methods accessible to scientists?
- how best to ensure that analyses are reproducible?
- + how best to enable transparent communication and reuse of analyses?

Vision

Galaxy is an open, Web-based platform for accessible, reproducible, and transparent computational biomedical research

What is Galaxy?

GUI for genomics

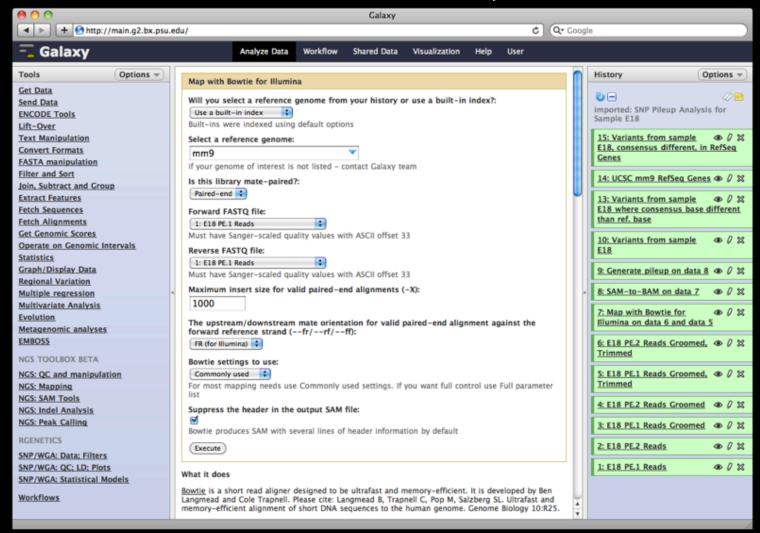
for complete analyses: analyze, visualize, share, publish

A free (for everyone) web service integrating a wealth of tools, compute resources, terabytes of reference data and permanent storage

Open source software that makes integrating your own tools and data and customizing for your own site simple



Accessibility



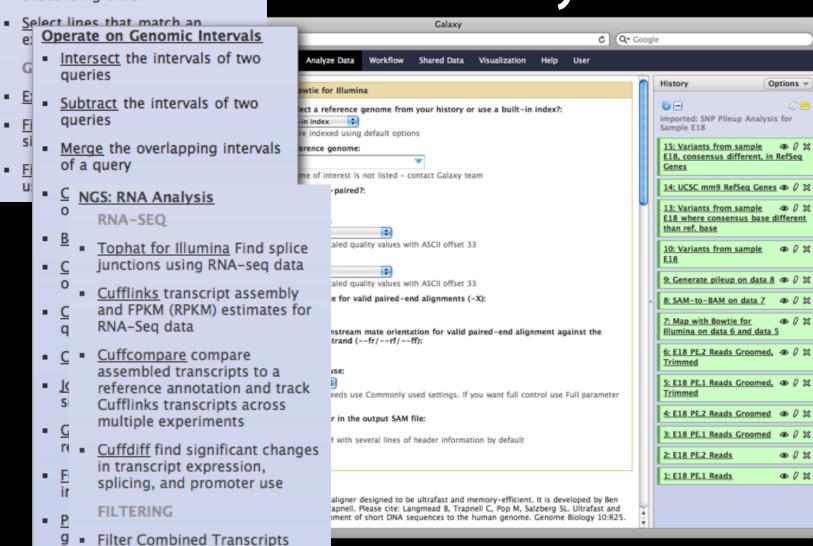
Filter and Sort

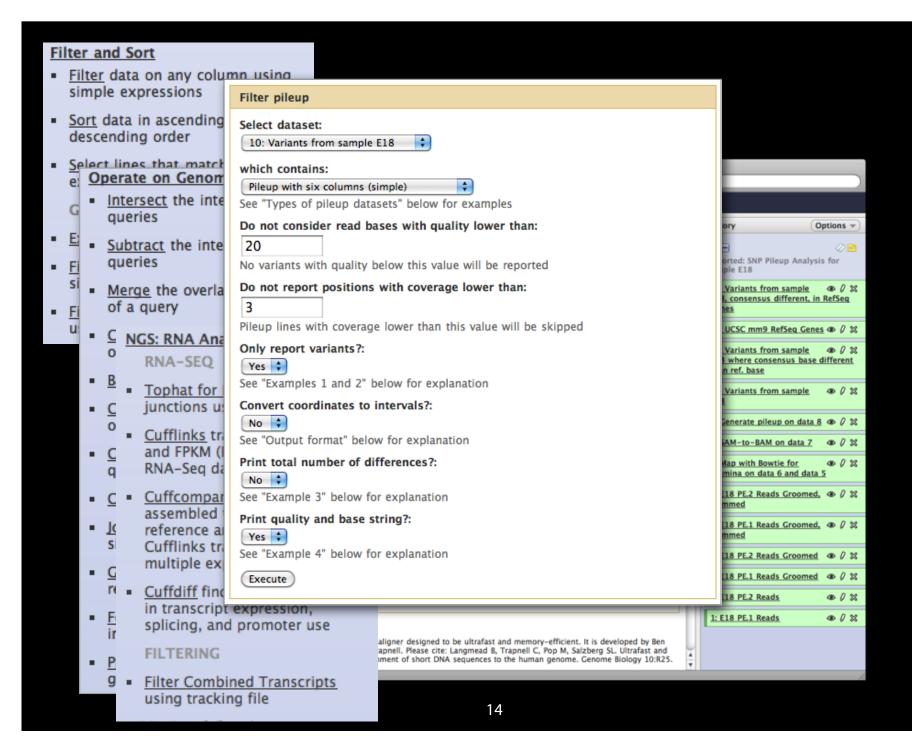
 <u>Filter</u> data on any column using simple expressions

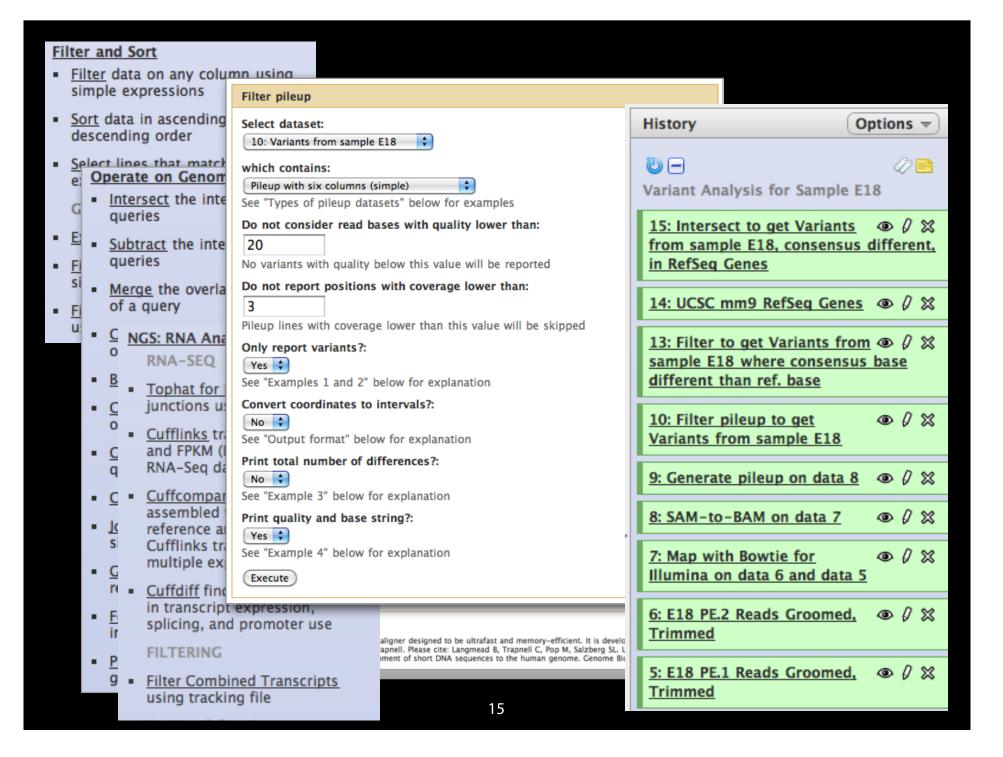
using tracking file

 Sort data in ascending or descending order

Accessibility







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Galaxy Accessibility

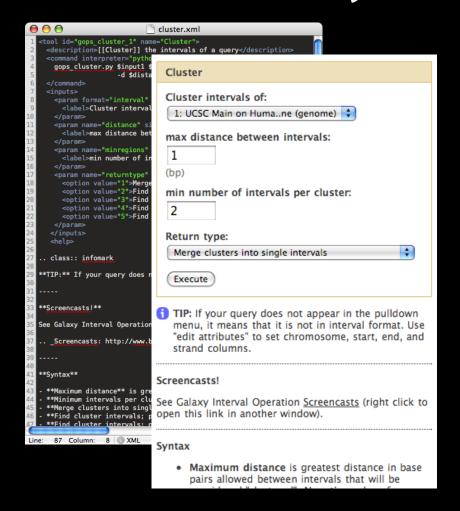
Only a Web browser is required

Standardization

+ tools, parameters, outputs all look the same

Easy to use output of one tool as input for another tool

Accessibility for Tool Developers



Defined via abstract interface:

- inputs & outputs
- parameters
- how to generate command line

As simple as possible but allows for rigorous

NGS: QC and manipulation ILLUMINA DATA

- FASTQ Groomer convert between various FASTQ quality formats
- <u>FASTQ splitter</u> on joined paired end reads
- <u>FASTQ joiner</u> on paired end reads
- <u>FASTQ Summary Statistics</u> by column

ROCHE-454 DATA

- Build base quality distribution
- · Select high quality segments
- Combine FASTA and QUAL into FASTO

AB-SOLID DATA

- Convert SOLiD output to fastq
- Compute quality statistics for SOLID data
- <u>Draw quality score boxplot</u> for SOLID data

GENERIC FASTQ MANIPULATION

- <u>Filter FASTQ</u> reads by quality score and length
- FASTQ Trimmer by column
- FASTQ Quality Trimmer by sliding window
- FASTO Masker by quality score

FASTQC: FASTQ/SAM/BAM

 <u>Fastqc: Fastqc QC</u> using FastQC from Babraham

Evolution

Metagenomic analyses
Human Genome Variation
EMBOSS

NGS TOOLBOX BETA

NGS: QC and manipulation

ILLUMINA

- · Map with Bowtie for Illumina
- Map with BWA for Illumina ROCHE-454
- <u>Lastz</u> map short reads against reference sequence
- Megablast compare short reads against htgs, nt, and wgs databases
- Parse blast XML output
 AB-SOLID
- Map with Bowtie for SOLiD

NGS: SAM Tools

NGS: Indel Analysis

NGS: Peak Calling

NGS: RNA Analysis

RGENETICS

SNP/WGA: Data; Filters SNP/WGA: QC; LD; Plots SNP/WGA: Statistical Models

Workflows

NGS: Picard (beta)

QC/METRICS FOR SAM/BAM

- BAM Index Statistics
- Sam/bam Alignment Summary Metrics
- Sam/bam GC Bias Metrics
- Estimate Library Complexity
- Insertion size metrics for PAIRED data
- Sam/bam Hybrid Selection <u>Metrics</u> For (eg exome) targeted data

BAM/SAM CLEANING

- Add or Replace Groups
- Reorder SAM
- Replace Sam Header
- <u>Paired Read Mate Fixer</u> for paired data
- Mark Duplicate reads

NGS: GATK Tools

REALIGNMENT

- Realigner Target Creator for use in local realignment
- Indel Realigner perform local realignment

BASE RECALIBRATION

- Count Covariates on BAM files
- Table Recalibration on BAM files
- Analyze Covariates perform local realignment

GENOTYPING

 Unified Genotyper SNP and indel caller

NGS: SAM Tools

NGS: Indel Analysis

- Filter Indels for SAM
- Extract indels from SAM
- Indel Analysis

NGS: Peak Calling

- MACS Model-based Analysis of ChIP-Seq
- GeneTrack indexer on a BED file
- <u>Peak predictor</u> on GeneTrack index

NGS: RNA Analysis

RNA-SEO

- Tophat Find splice junctions using RNA-seg data
- <u>Cufflinks</u> transcript assembly and FPKM (RPKM) estimates for RNA-Seg data
- <u>Cuffcompare</u> compare assembled transcripts to a reference annotation and track Cufflinks transcripts across multiple experiments
- <u>Cuffdiff</u> find significant changes in transcript expression, splicing, and promoter use

FILTERING

 <u>Filter Combined Transcripts</u> using tracking file

RGENETICS

Amplification

Many tools available in a single place means that tools can be combined in novel ways

Users, developers, community benefit

Reproducibility

Reproducibility in Genomics

18 *Nat. Genetics* experiments in microarray gene expression

<50% of reproducible

Problems

- missing data (38%)
- missing software, hardware details (50%)
- missing method, processing details (66%)

Ioannidis, J.P.A. et al. "Repeatability of published microarray gene expression analyses." Nat Genet 41, 149-155 (2009)

Reproducibility in Genomics

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- missing method, processing details (66%)

loannidis, J.P.A. et al. "Repeatability of published microarray gene expression analyses." Nat Genet 41, 149-155 (2009)

14 re-sequencing experiments in *Nat. Genetics, Nature, Science*

0% reproducible?

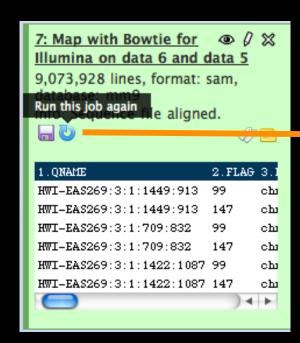
Problems

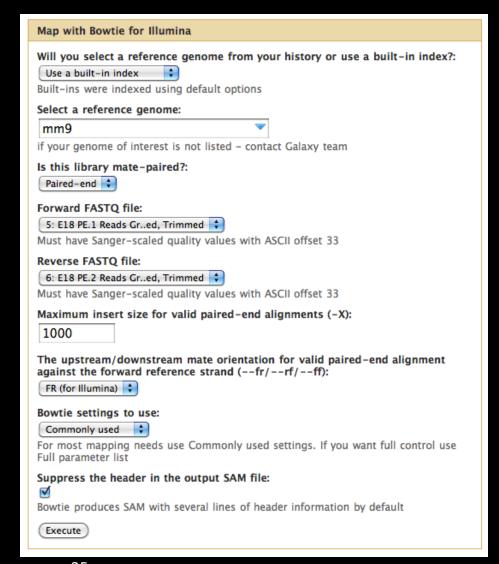
- missing primary data (50%)
- tools unavailable (50%)
- missing parameter setting, tool versions (100%)

"Devil in the details," Nature, vol. 470, 305-306 (2011).

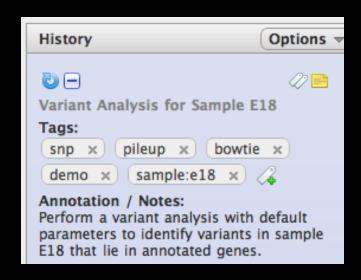
Metadata = Reproducibility

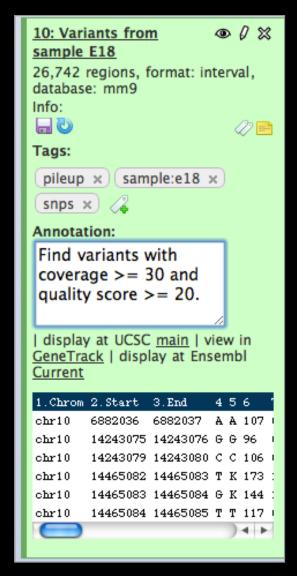
Automatic Metadata



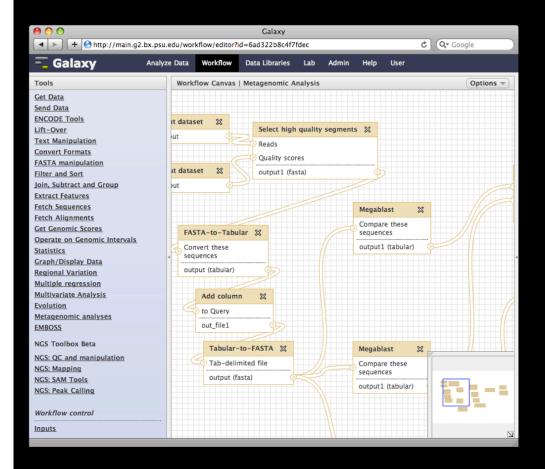


User Metadata





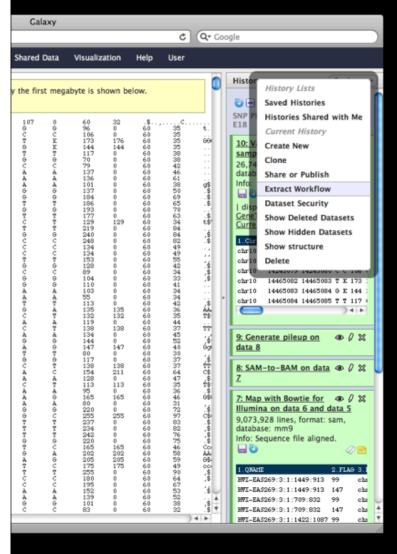
Galaxy Workflow System

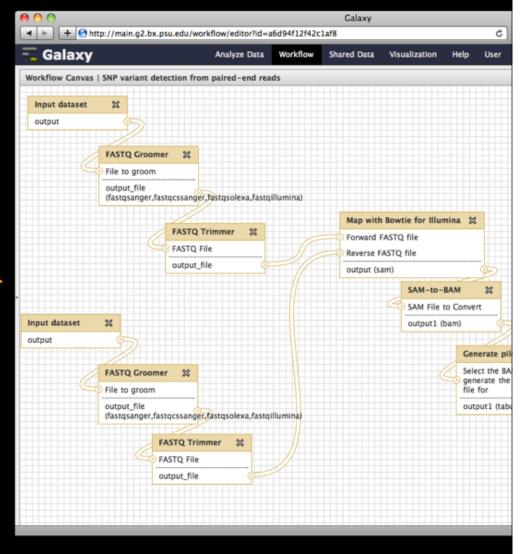


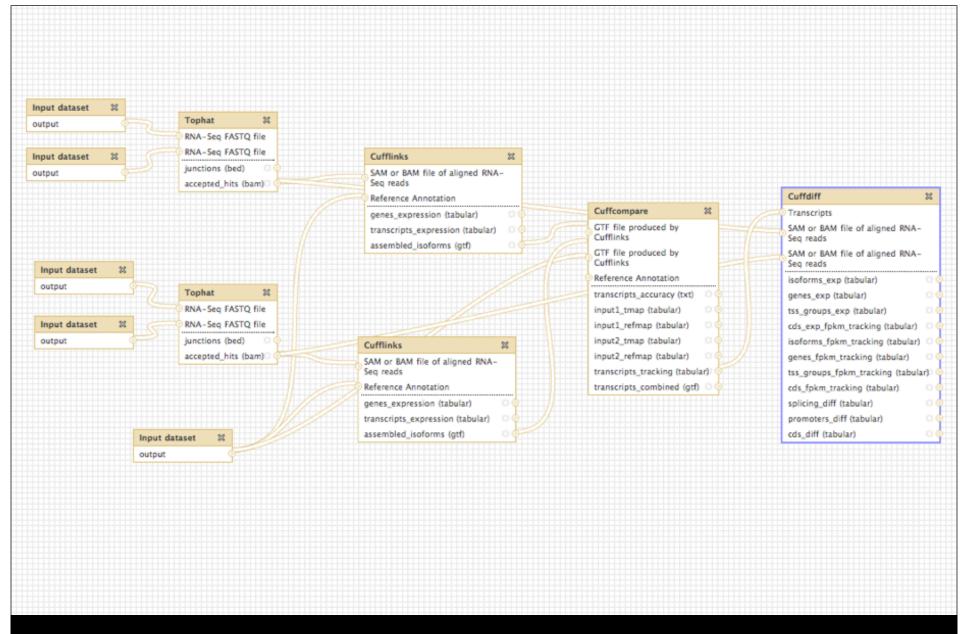
Workflows can be constructed from scratch *or* extracted from existing analysis histories

Facilitate reuse and provide precise reproducibility of a complex analysis

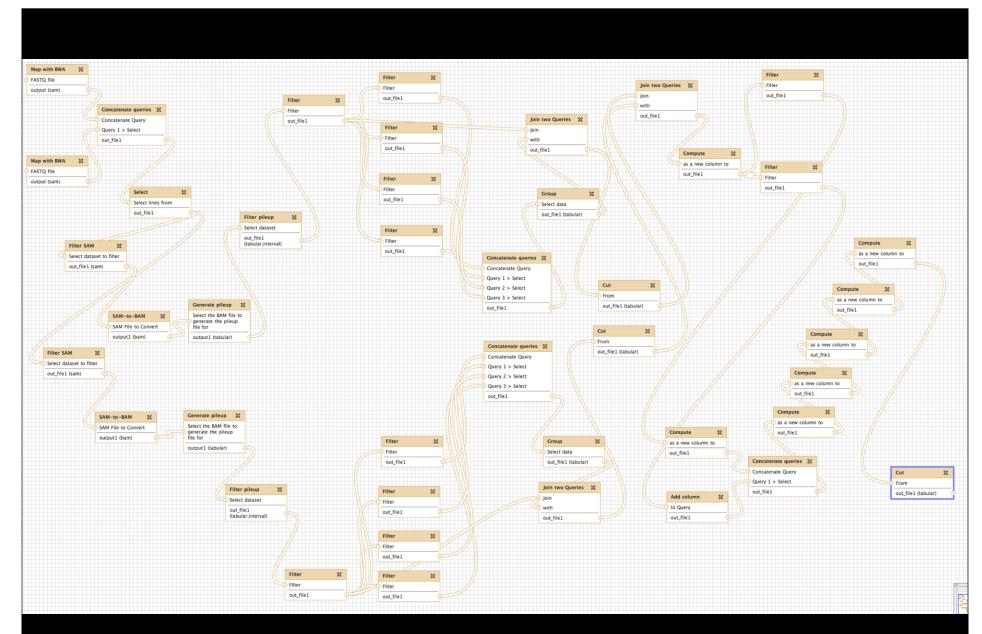
Galaxy Workflows







Example: Workflow for differential expression analysis of RNA-seq using Tophat/Cufflinks tools



Example: Diagnosing low-frequency heterosplasmic sites in two tissues from the same individual



Everything can be shared

Sharing and Publishing History 'Variant Analysis for Sample E18'

Making History Accessible via Link and Publishing It

This history accessible via link and published.

Anyone can view and import this history by visiting the following URL:

http://main.q2.bx.psu.edu/u/jgoecks/h/variant-analysis-for-sample-e18

This history is publicly listed and searchable in Galaxy's Published Histories section.

You can:

Unpublish History

Removes history from Galaxy's Published Histories section so that it is not publicly listed or searchable.

Disable Access to History via Link and Unpublish

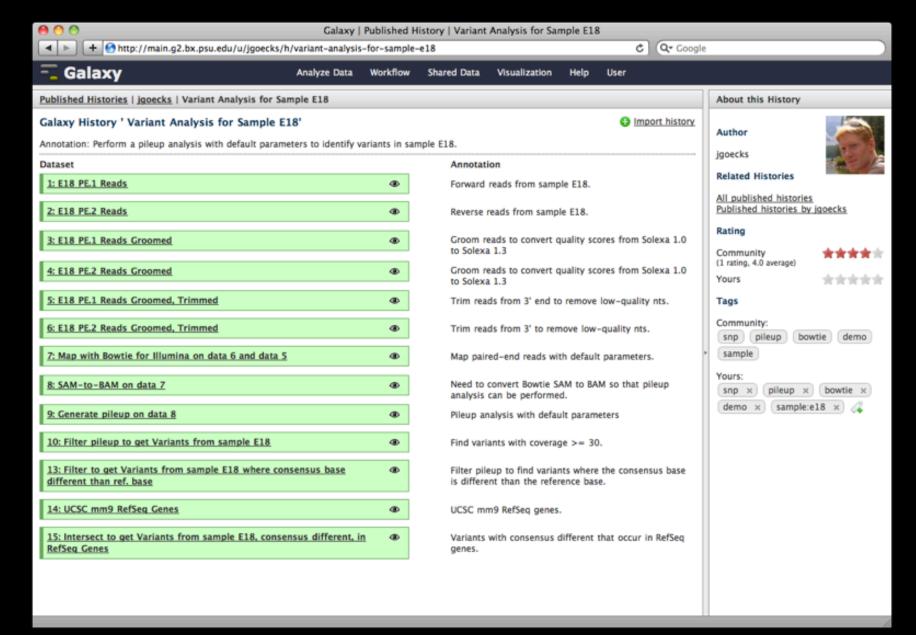
Disables history's link so that it is not accessible and removes history from Galaxy's <u>Published Histories</u> section so that it is not publicly listed or searchable.

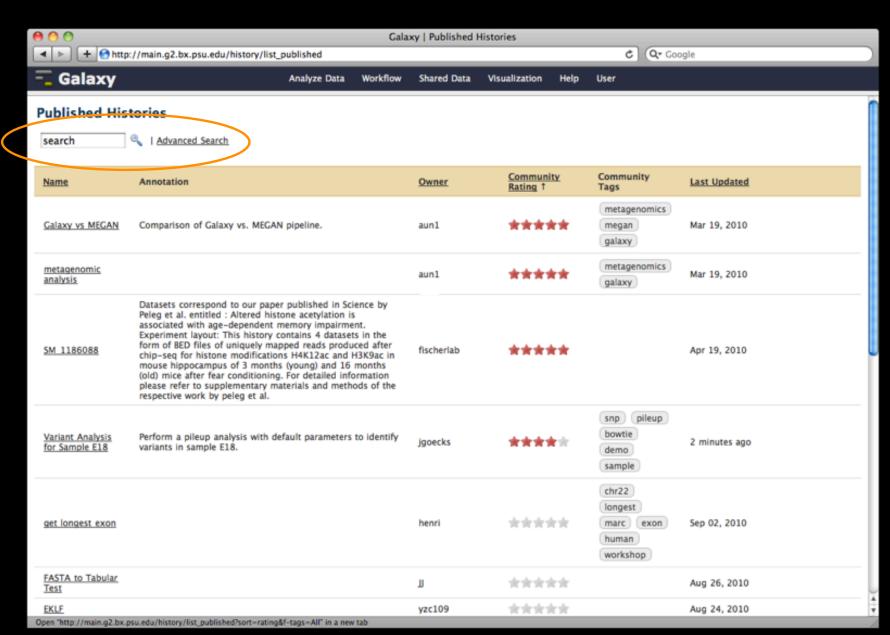
Sharing History with Specific Users

You have not shared this history with any users.

Share with a user

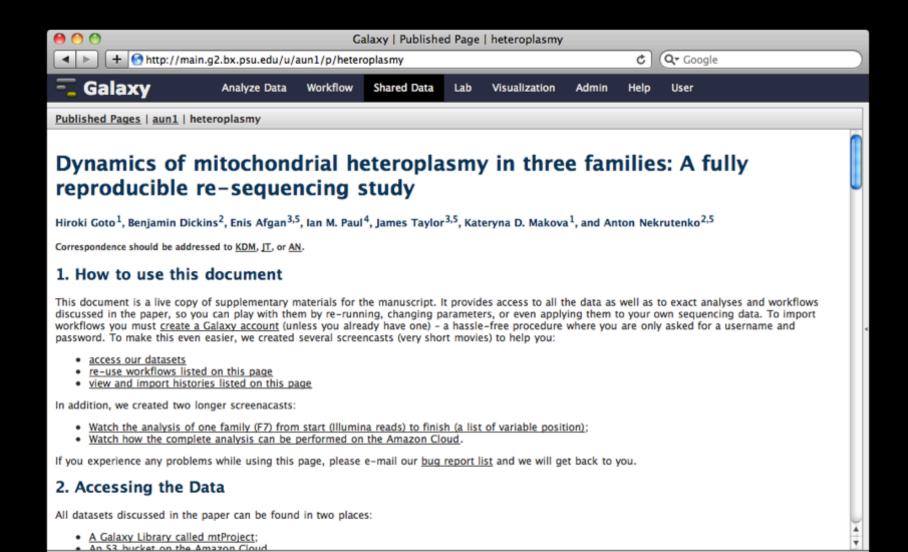
Back to Histories List

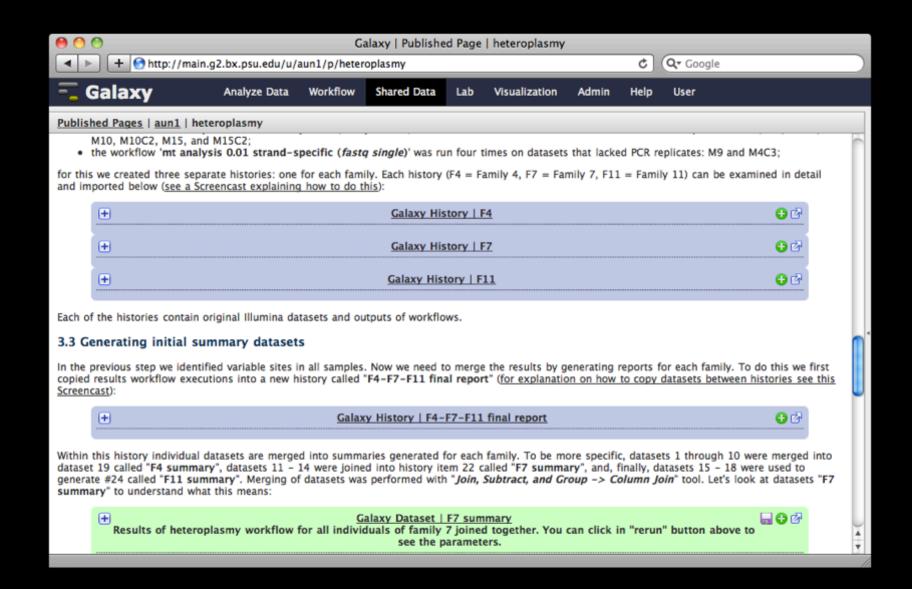




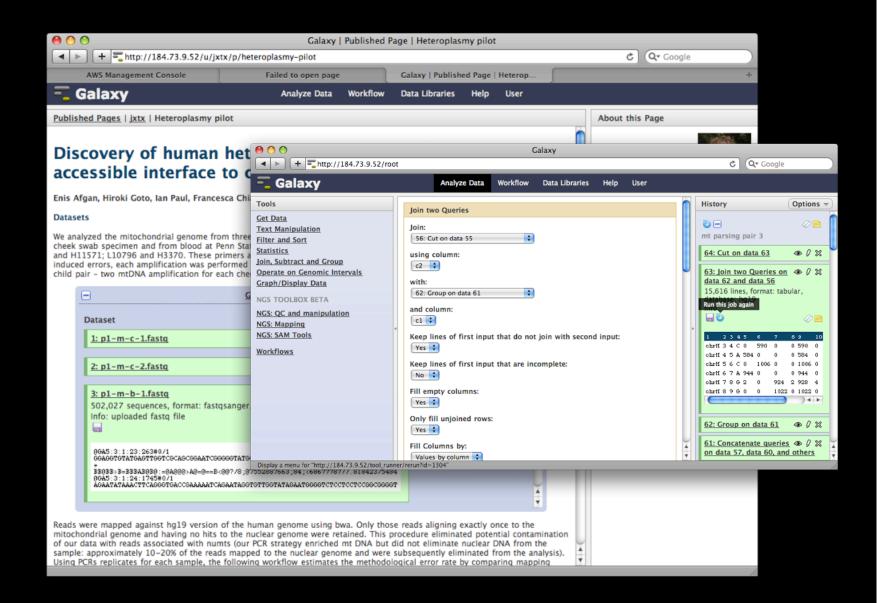
Galaxy Pages

A web-based, interactive medium for presenting all aspects of an analysis: data, methods, visualization, and results

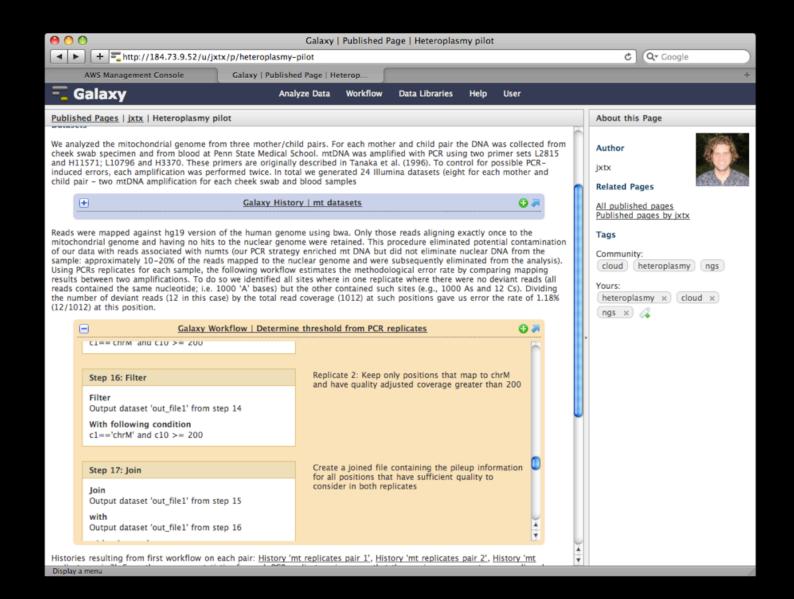




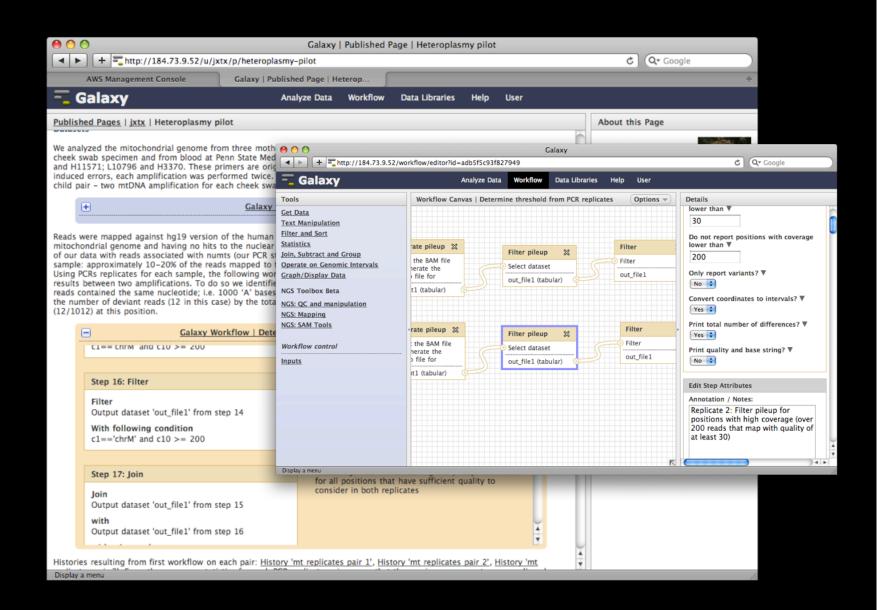
Actual histories and datasets directly accessible from the text



Histories can be imported and the exact parameters inspected



Workflows and other entities can also be embedded



And imported for inspection, verification, and reuse

The Power of Galaxy Publishing

Galaxy's publishing features facilitate access and reproducibility without any extra leg work

One click grants access to the *actual analysis* you performed to generate your original results

- not just data access, the full pipeline + annotations
- anyone can import your work and immediately reproduce or build on it





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OPEN ACCESS ARTICLE

This Article

Published in Advance October 9, 2009, doi: 10.1101/gr.094508.109

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October 2010, 20 (10)



+ From the Cover

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Do you know what your current research approach is

Windshield splatter analysis with the Galaxy metagenomic pipeline

Sergei Kosakovsky Pond 1,2,6,9, Samir Wadhawan 3,6,7, Francesca Chiaromonte⁴, Guruprasad Ananda^{1,3}, Wen-Yu Chung^{1,3,8}, James Taylor 1,5,9, Anton Nekrutenko 1,3,9 and The Galaxy Team 1

+ Author Affiliations

Abstract

000

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 erganic matter collected by a

Footpotes

[Supplemental material is available online at http://www.genome.org. All data and tools described in this manuscript can be downloaded or used directly at http://galaxyproject.org. Exact analyses and workflows used in this paper are available at http://usegalaxy.org/u/aun1/p/windshield-splatter.]

Article published online before print. Article and publication date are at http://www.genome.org/cgi/doi/10.1101/gr.094508.109.

Three Ways to Use Galaxy

1. Public Website (http://usegalaxy.org)

2. Download and run locally

3. Run on the cloud

Galaxy main site (http://usegalaxy.org)

Public Website, anybody can use

~500 new users per month, ~100 TB of user data, ~130,000 analysis jobs per month, every month is our busiest month ever...

Will continue to be maintained and enhanced, but with limits and quotas

Centralized solution cannot scale to meet data analysis demands

Download and Run Locally

No configuration needed but everything can be configured

- tools
- computing cluster integration
- proxy server and authentication

Prominent local installations at:

- Cold Spring Harbor Lab
- Dept. of Energy's Joint Genome Institute
- Harvard School of Public Health
- U of Texas System
- Netherlands Bioinformatics Center
- Oxford College

Requires computing resources, technical expertise, and maintenance

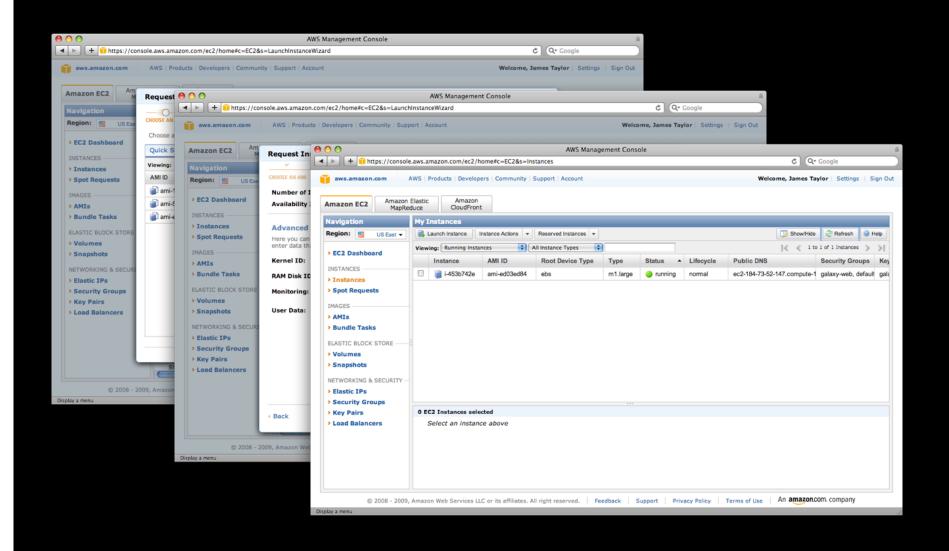
Galaxy on the Cloud

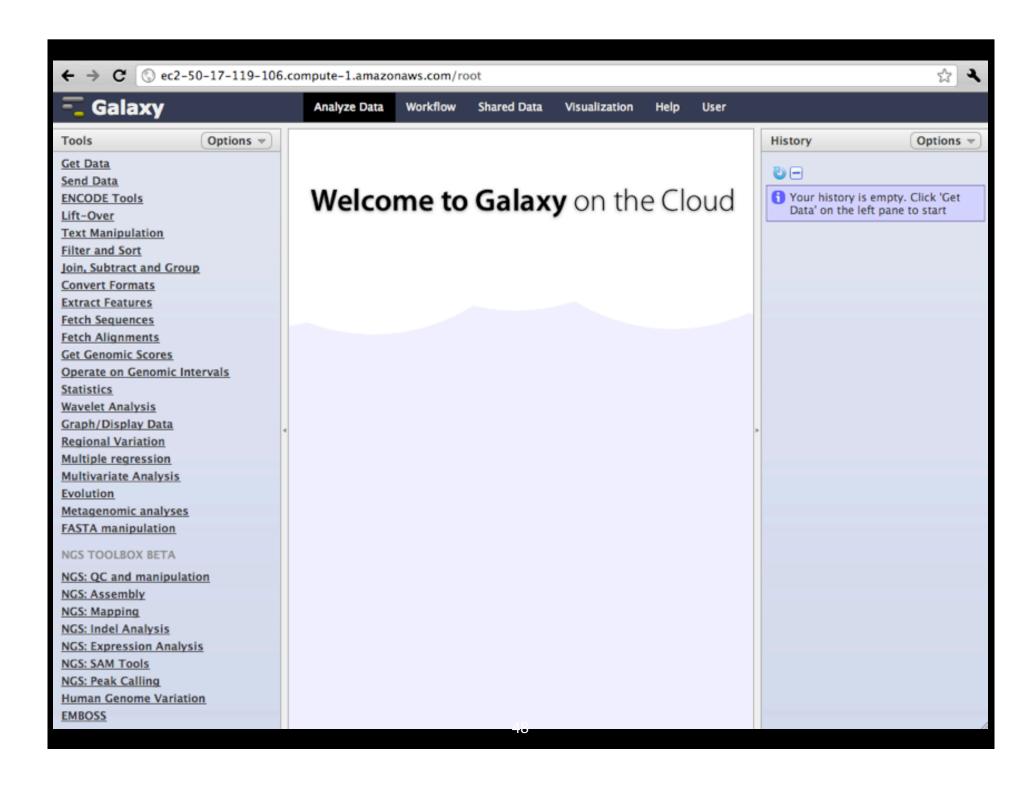
For extended or particular resource needs

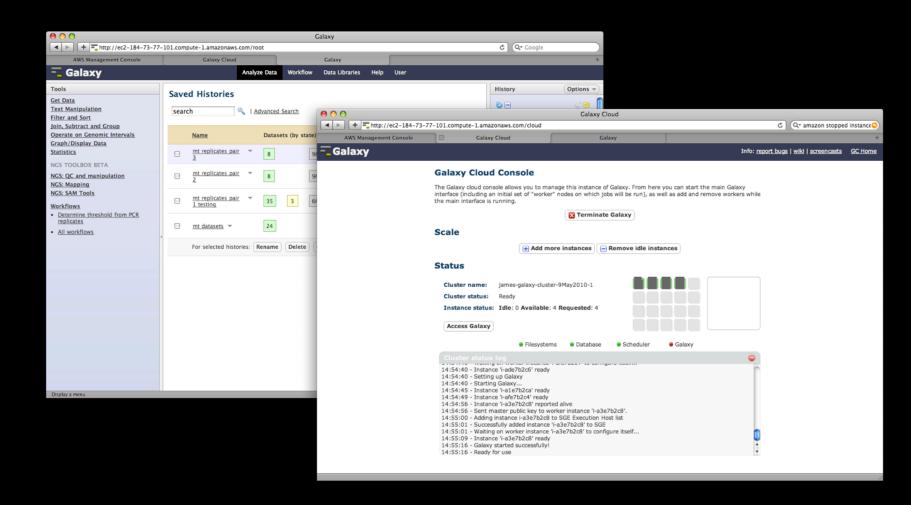
- customization necessary
- oscillating data volume

For when informatics expertise or infrastructure is limited

Using Amazon EC2: Startup in 3 steps







Like any other Galaxy instance plus:

- additional compute nodes acquired and released automatically as needed
- can share or publish an instance

Challenges Going Forward

Promoting community involvement

- tools, assays, analyses growing too fast for us alone
- facilitate community contributions and usage of contributions

Scaling to many, many Galaxies

- + moving objects between Galaxies while ensuring reproducibility
- enabling users to find useful "stuff"

Novel application areas

genomics ideal application area -- what next?





Enis Afgan



Dannon Baker



Dave Clements



Jeremy Goecks



James Taylor



PENNSTATE.

Dan Blankenberg



Nate Coraor



Jennifer Jackson



Greg von Kuster

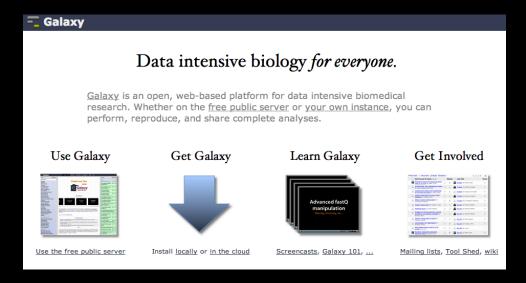


Anton Nekrutenko

Supported by the **NHGRI** (HG005542, HG004909, HG005133), **NSF** (DBI-0850103), Penn State University, Emory University, and the Pennsylvania Department of Public Health

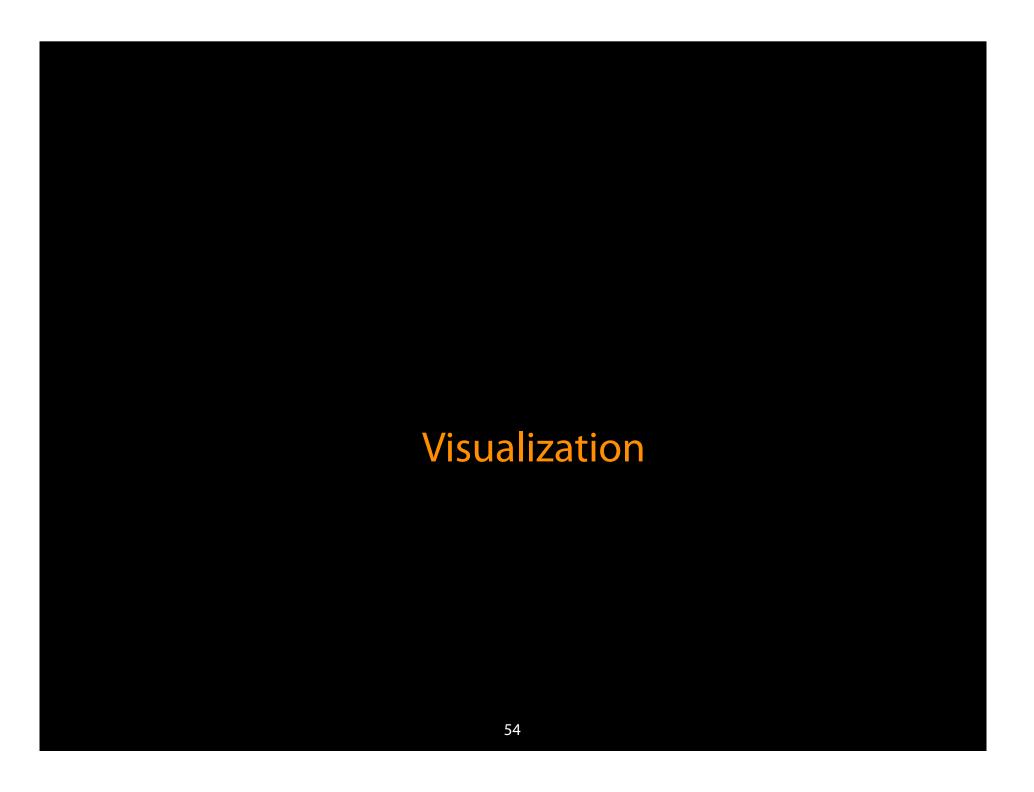
Thanks! Questions?

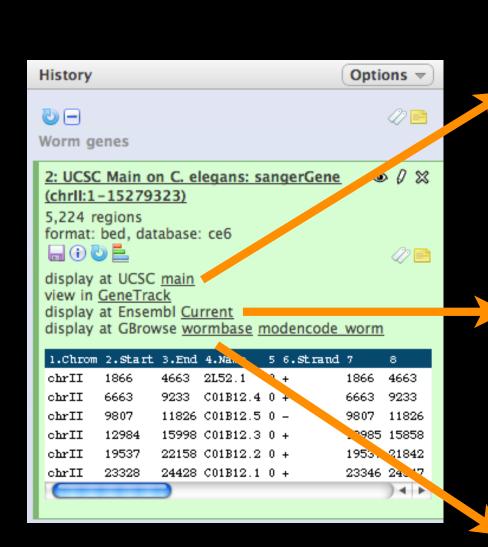
http://galaxyproject.org

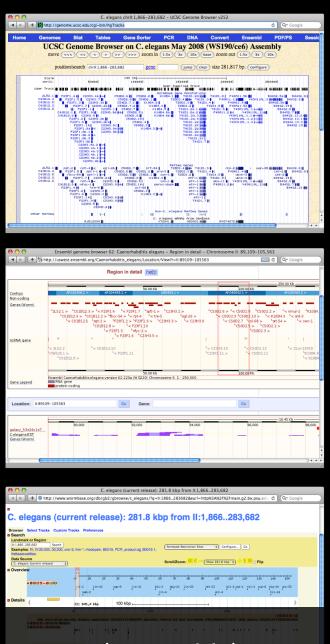


Galaxy publications: http://galaxyproject.org/wiki/Citing Galaxy is hiring! http://galaxyproject.org/wiki/GalaxyisHiring

jeremy.goecks@emory.edu







Integration with many existing browsers (extensible)

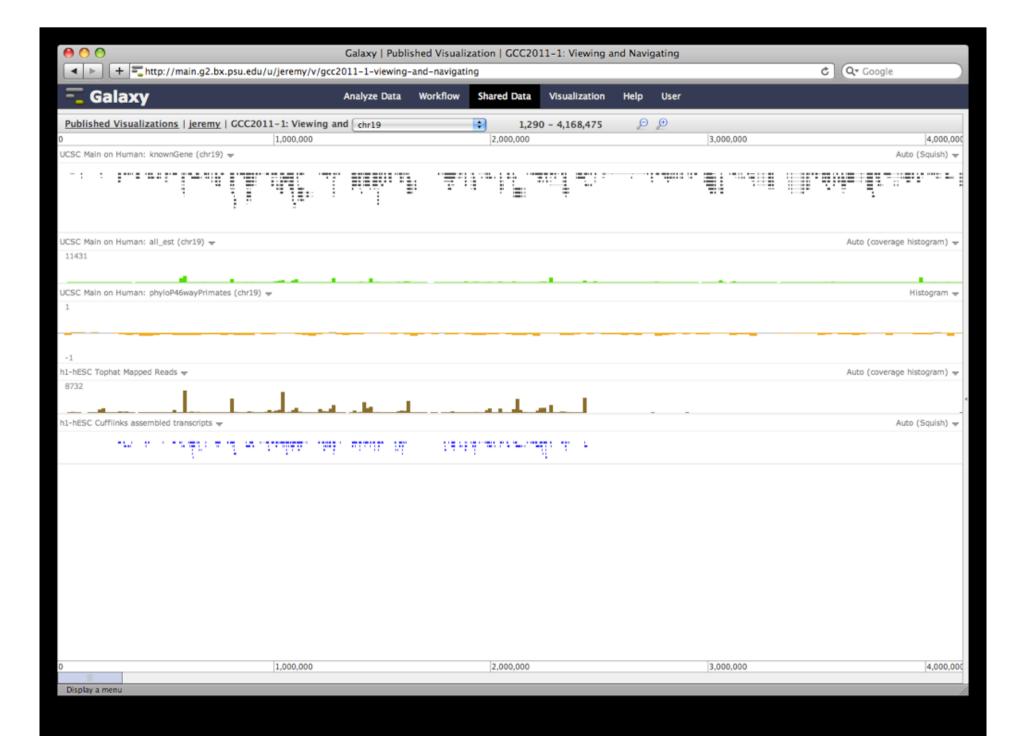
Galaxy

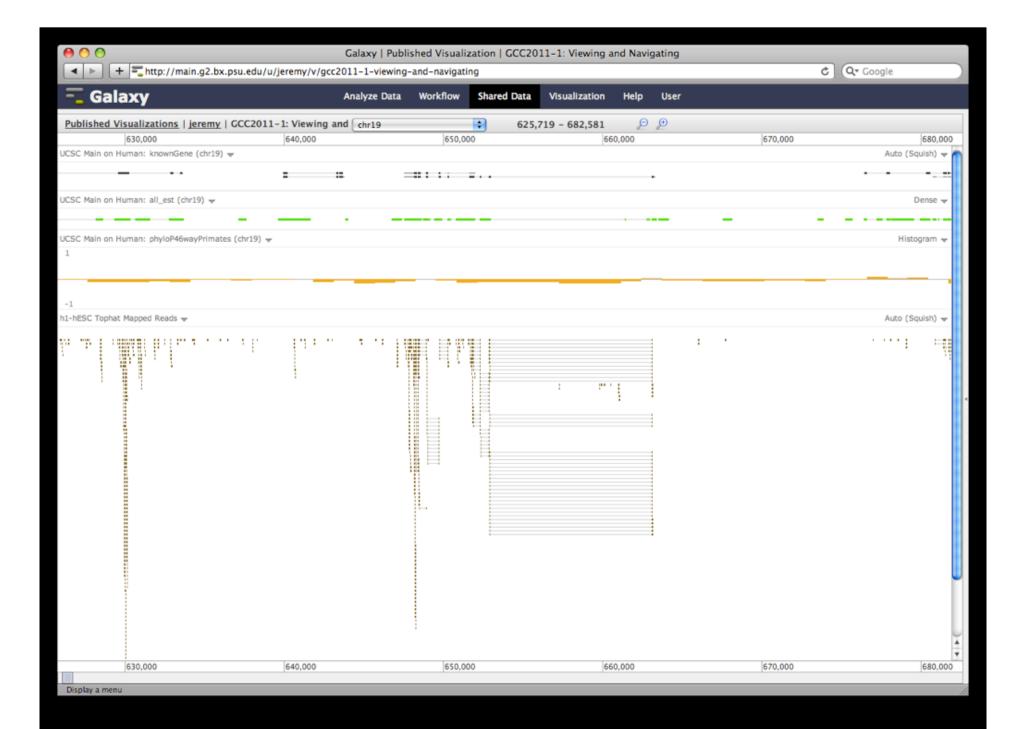
- tool integration framework
- heavy focus on usability
- sharing, publication framework

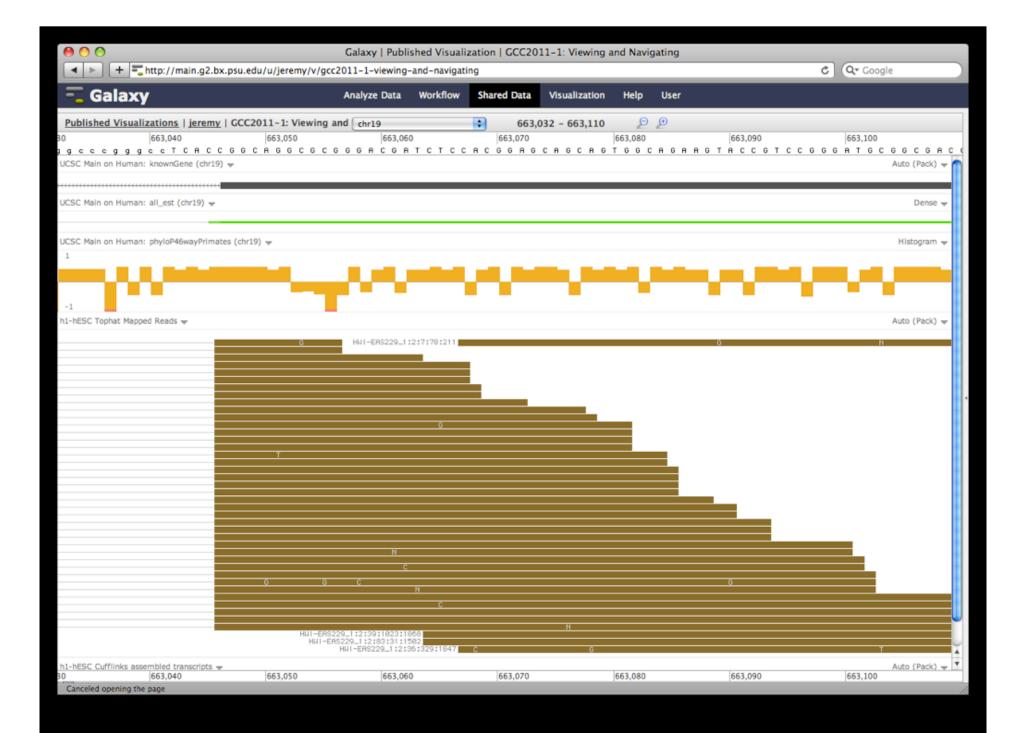
Genome Browser

- physical depiction of data
- visually identify correlations
- find interesting regions, features

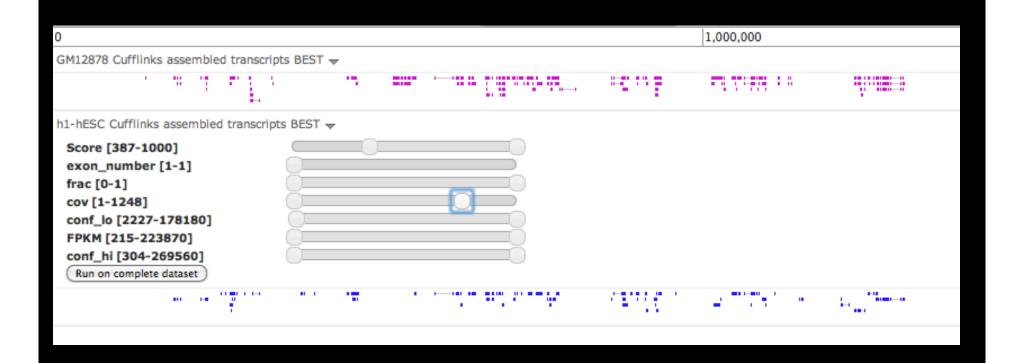




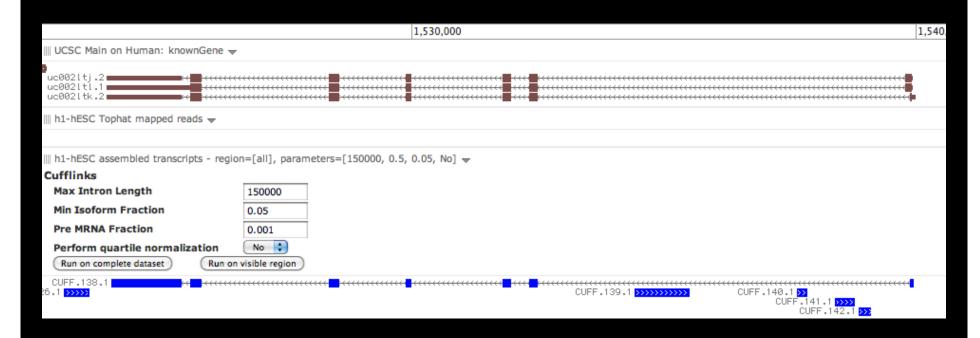




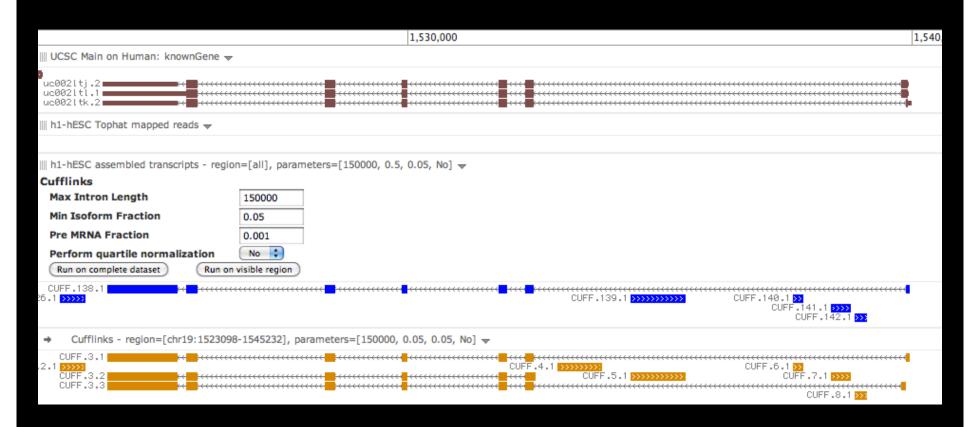
Dynamic Filtering



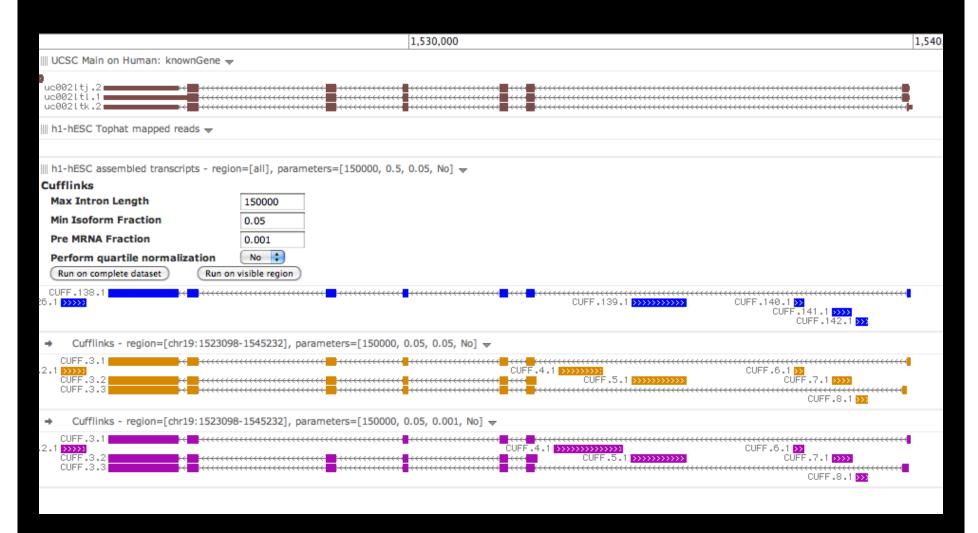
Integrating Tools and Visualization



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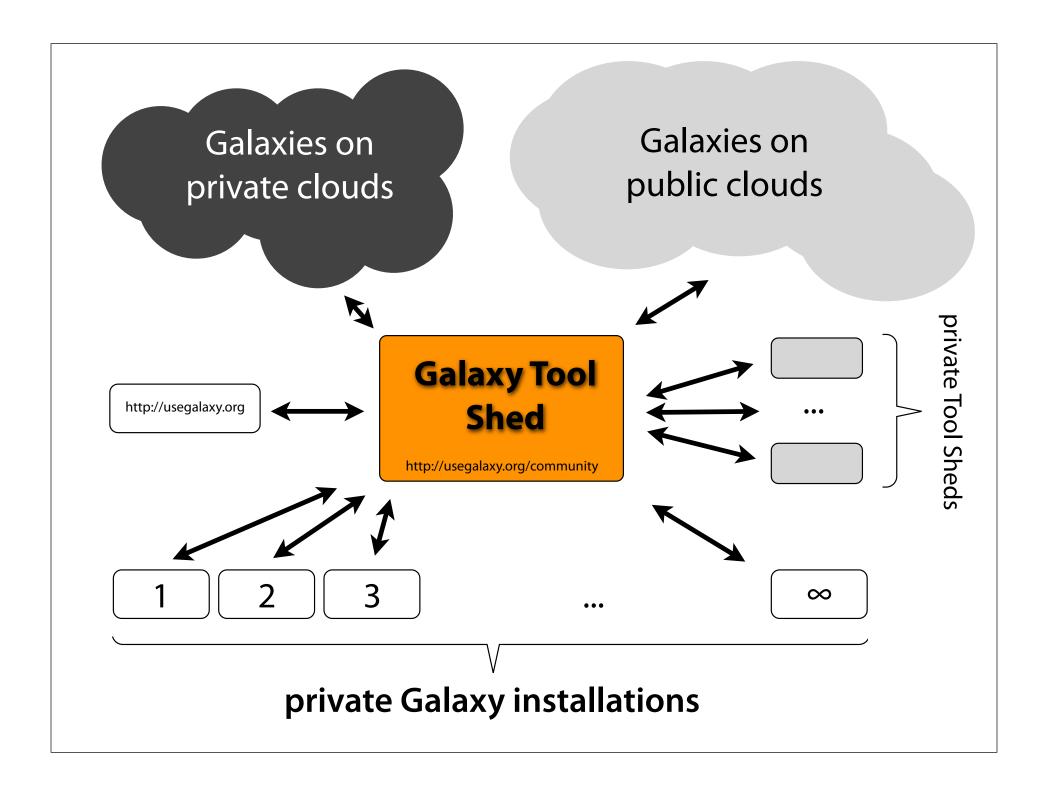
Scaling Galaxy: two distinct problems

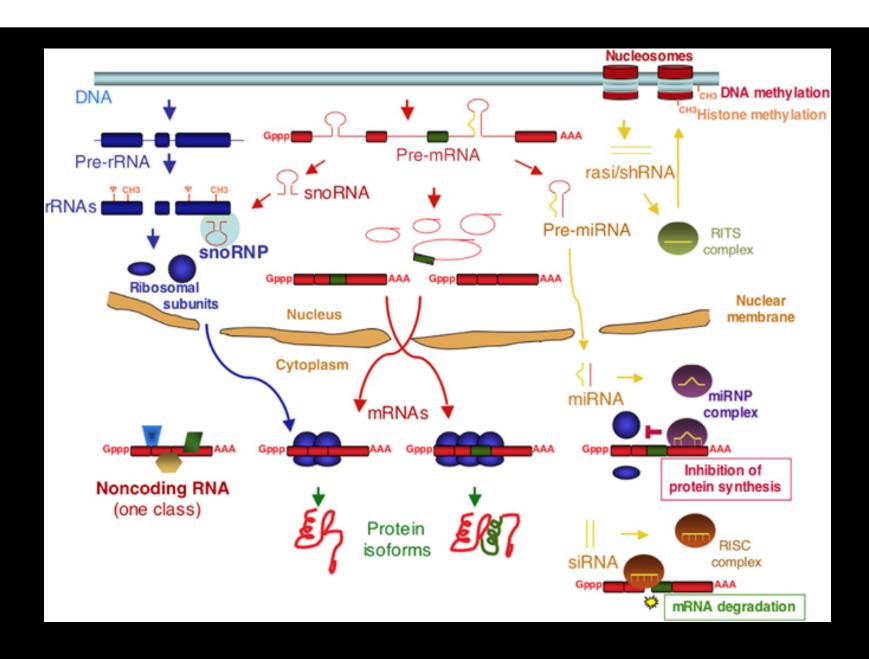
So much data, not enough infrastructure

 solution: encourage local Galaxy instances, cloud Galaxy, support increasingly decentralized model

So many tools and workflows, not enough manpower

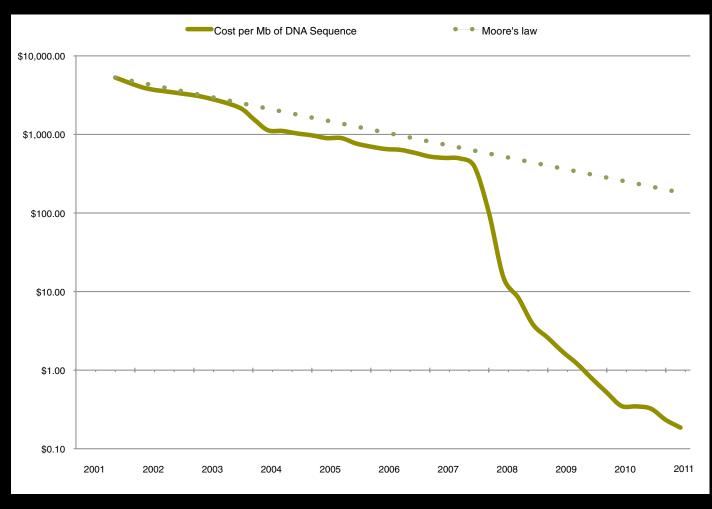
 focus on building infrastructure to allow community to integrate and share tools, workflows, and best practices





"The expanding transcriptome: the genome as the 'Book of Sand'", Soares and Valcarcel, *The EMBO Journal* (2006) 25

Trends in Genomics



Sboner et al. Genome Biology 2011, 12:125