http://bit.ly/gxyeshg2015

Variant Analysis with Galaxy

ESHG 2015

Glasgow, United Kingdom
6 June 2015

Dave Clements
Galaxy Project
Johns Hopkins University

Outline

Introduction: The Galactic Landscape

Variant Analysis: A worked example

Variant Analysis: Other options

Galaxy: Resources and Community

Done

Slides at http://bit.ly/gxyeshg2015

Goals

Provide a basic introduction to using Galaxy for bioinformatic analysis.

Demonstrate how Galaxy can help you explore and learn options, perform analysis, and then share, repeat, and reproduce your analyses.

Not Goals

This workshop will *not* cover

- details of how tools are implemented, or
- new algorithm designs, or
- which variant caller or assembler or mapper or peak caller or ... is best for you.

What is Galaxy?

Data integration and analysis platform that emphasizes accessibility, reproducibility, and transparency

A free (for everyone) web server

Open source software

These options result in several ways to use Galaxy

http://galaxyproject.org

Galaxy is available online, for free

http://usegalaxy.org

As a free (for everyone) web server integrating a wealth of tools, compute resources, petabytes of reference data and permanent storage

However, ***a centralized solution cannot support the different analysis needs of the entire world.***

Galaxy is available as Open Source Software

Galaxy is installed in locations around the world.

Some of them are free for anyone to use too.

http://getgalaxy.org

bit.ly/gxyServers

Galaxy is available on the Cloud

openstack™
CLOUD SOFTWARE

amazon web services™

OpenNebula.org
The Open Source Toolkit for Cloud Computing

globus genomics

http://aws.amazon.com/education
http://globus.org/
http://wiki.galaxyproject.org/Cloud

We are using the cloud today.

Galaxy is available with Commercial Support

A ready-to-use appliance
(BioTeam)

Cloud-based solutions
(ABgenomica, AIS, GenomeCloud)

Consulting & Customization
(BioTeam, Deena Bioinformatics)

Training
(OpenHelix)

SLIPSTREAM APPLIANCE GALAXY EDITION
Galaxy made easy.

abgenomica

AIS ENTERPRISE-CLASS DATA CENTER SERVICES

GenomeCloud

BIOTEAM

OpenHelix™

Galaxy Project: Further reading & Resources

http://galaxyproject.org

http://usegalaxy.org

http://getgalaxy.org

http://wiki.galaxyproject.org/Cloud

http://bit.ly/gxychoices

Outline

Introduction: The Galactic Landscape

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Slides at http://bit.ly/gxyeshg2015

Galaxy | Analyze Data | Workflow | Shared Data | Visualization | Cloud | Help | User

Published Pages | galaxyproject | Galaxy 101 NGS: Introduction to Polymorphism Detection via Variant Analysis

Galaxy 101 NGS: Introduction to Polymorphism Detection via Variant Analysis

Heteroplasmy: Mother-Child mtDNA Variant Polymorphism

• heteroplasmy • ismb2010-demo

This tutoria

- Import Sequence
- Interpret FASTQ
- Execute a series
- Execute one com
- Filter key results.

What is Hete

The heteroplastom
mtDNA heteroplast
research in the fiel

Being heteroplasto

- Go ahead, start w

Experiment

Import child and m
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and FreeBayes. Not
Variant Annotator

- Explore the resul
- Questions:
  - Can you ident
  - Can you ident
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  - How would yo
    mtDNA (per i

* Source at Illumina

Input NGS Dat

Galaxy | Analyze Data | Workflow | Shared Data | Visualization | Cloud | Help | User

Published Pages | aun1 | heteroplasmy

Dynamics of mitochondrial heteroplasmy in three families investigated via a repeatable re-sequencing study

Hiroki Goto[1], Benjamin Dickins[2], Enis Afgan[3,5], Ian M. Paul[4], James Taylor[3,5], Kateryna D. Makova[1], and Anton Nekrutenko[2,5]

Published in Genome Biology on June 23, 2011

Correspondence should be addressed to KDM, JT, or AN.

1. How to

This document is
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access our datase
re-use workflows
view and import
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2. Access

All datasets discu

A Galaxy Library
An S3 bucket on
From there these
family is "F4", "F7
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table = count of

F1

F2

M9
blood
6,106,21

Goto *et al. Genome Biology* 2011, **12**:R59
http://genomebiology.com/2011/12/6/R59

Genome Biology

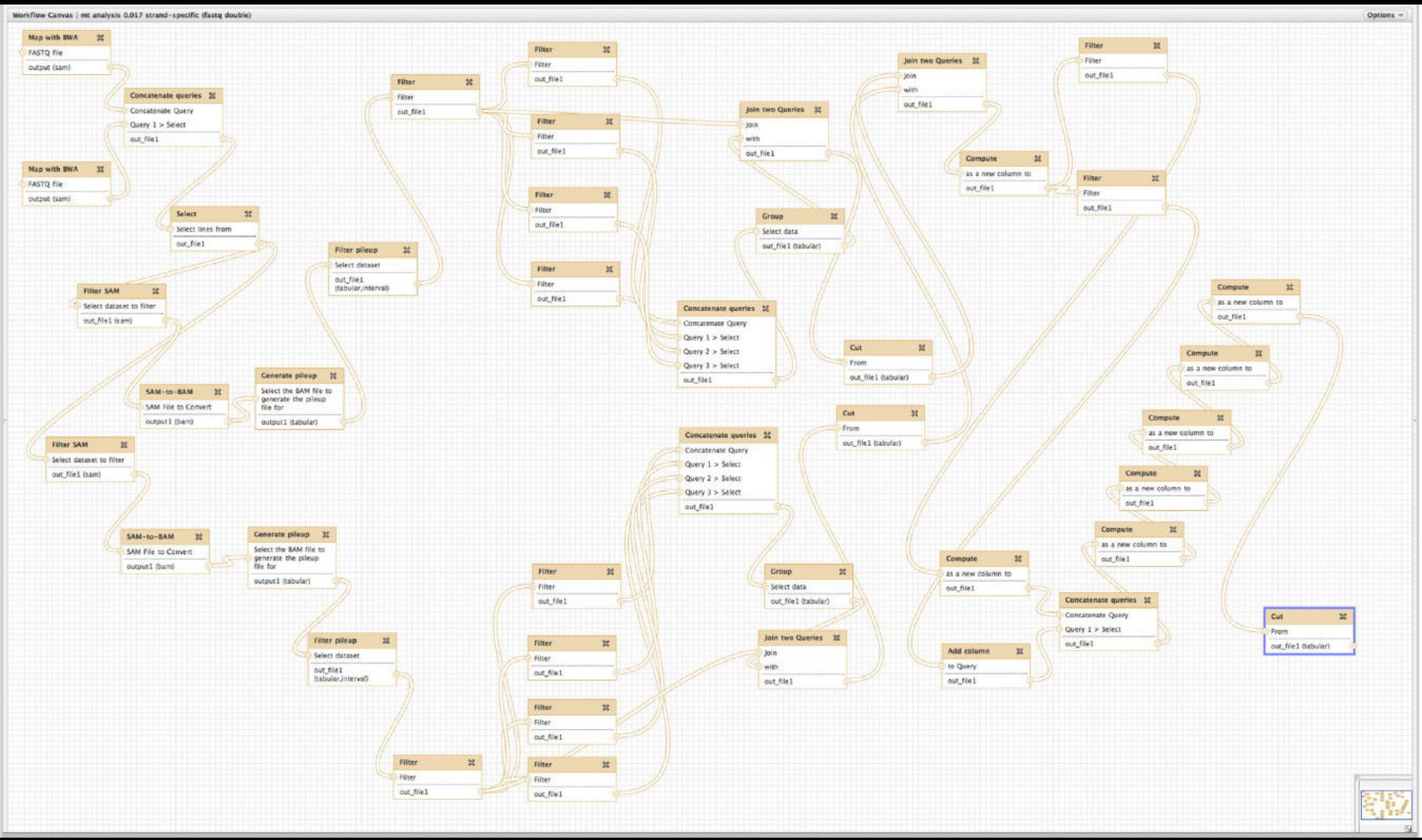
RESEARCH Open Access

Dynamics of mitochondrial heteroplasmy in three families investigated via a repeatable re-sequencing study

Hiroki Goto[1†], Benjamin Dickins[2†], Enis Afgan[3], Ian M Paul[4], James Taylor[3*], Kateryna D Makova[1*] and Anton Nekrutenko[2*]

Abstract

Background: Originally believed to be a rare phenomenon, heteroplasmy - the presence of more than one mitochondrial DNA (mtDNA) variant within a cell, tissue, or individual - is emerging as an important component of


Workflow Canvas | mt analysis 0.017 strand-specific (fastq double)
Options
Map with BWA
FASTQ file
output (sam)
Concatenate queries
Concatenate Query
Query 1 > Select
out_file1
Map with BWA
FASTQ file
output (sam)
Select
Select lines from
out_file1
Filter SAM
Select dataset to filter
out_file1 (sam)
SAM-to-BAM
SAM File to Convert
output1 (bam)
Generate pileup
Select the BAM file to generate the pileup file for
output1 (tabular)
Filter pileup
Select dataset
out_file1 (tabular,interval)
Filter
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out_file1
Join two Queries
join
with
out_file1
Concatenate queries
Concatenate Query
Query 1 > Select
Query 2 > Select
Query 3 > Select
out_file1
Group
Select data
out_file1 (tabular)
Cut
From
out_file1 (tabular)
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Concatenate Query
Query 1 > Select
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Filter
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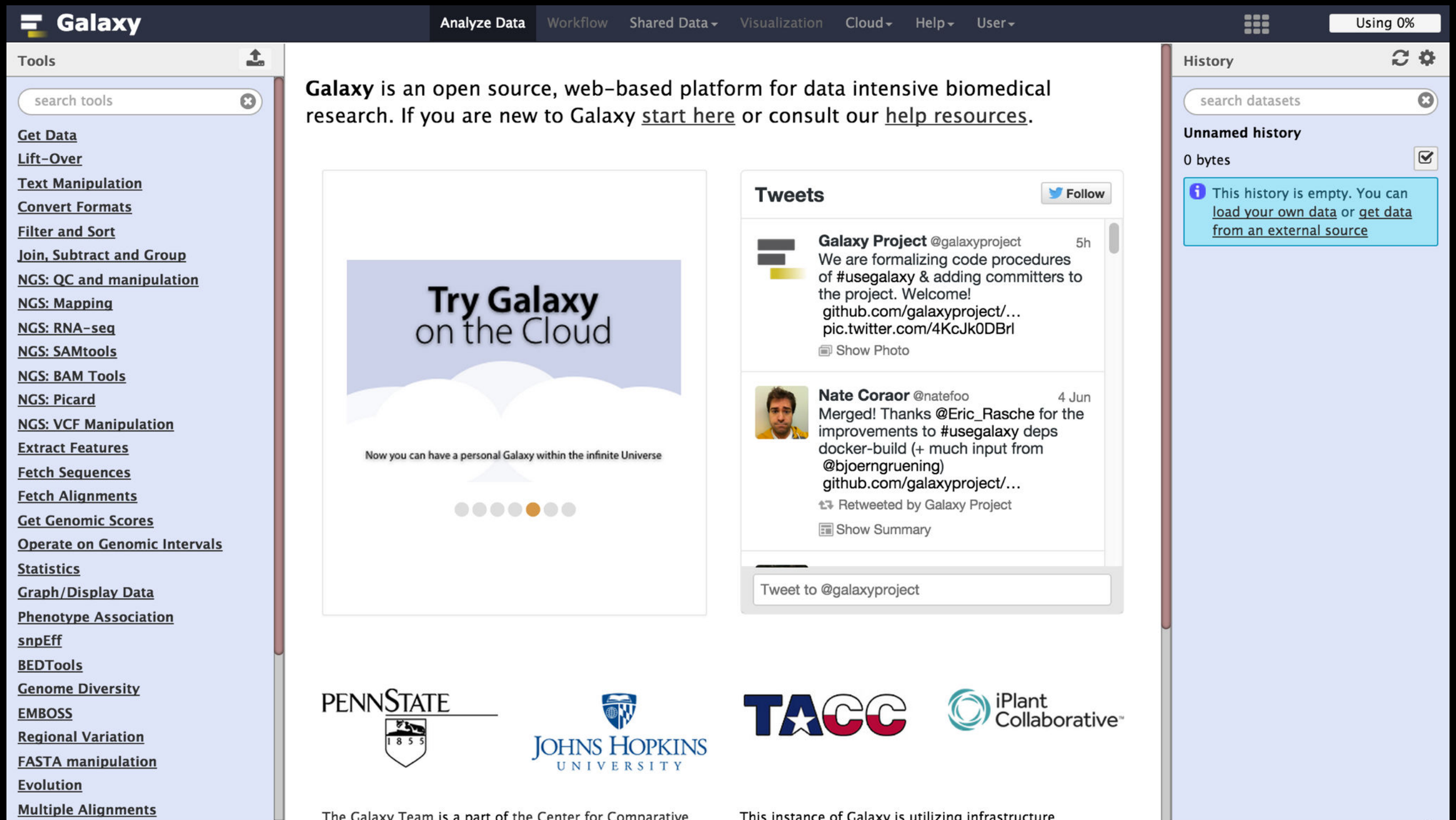
Group
Select data
out_file1 (tabular)
Join two Queries
join
with
out_file1

Variant analysis

Goal is to find variation in these individuals' mitochondria

I'm going to run this on usegalaxy.org



Variant analysis: First steps

Login / register

Start a new history

Shared Data → Data Libraries → Training → Heteroplasmy → M512

Select all

Import to current history

These are 4 paired end reads from mother and child, from blood or cheek.

Variant analysis: Quality Control

NGS QC is a workshop until itself

Should address it here.
We will actually get to it later.

See http://bit.ly/ngs101qc for more on QC.

Variant analysis: Mapping

Map mother and child paired end datasets using

NGS Mapping → Map with BWA-MEM

Reference genome → HG19

Select M512-bl_1 and M512-bl_2

Read Groups: basically metadata that allow tools to identify different samples / platforms / lanes / ...

Set Read group ID, Read Group Sample Name , Library Name, Platform unit

Variant analysis: Mapping

Now do it again, this time using
M512**C2**-bl_1 and M512**C2**-bl_2

Which can be kinda tedious.

Cheat #1: Rerun button

Variant analysis: Mapping

Now do it a third time, this time using
M512C2-**ch**_1 and M512C2-**ch**_2

Which now seems even more tedious.

Cheat #2: Multiple datasets button

Variant analysis: Mapping

Now do it a third time, this time using
M512C2-**ch**_1 and M512C2-**ch**_2

Which now seems even more tedious.

Cheat #2: Multiple datasets button

Why we didn't do that to begin with? *(and cheat #3, maybe)*

Variant analysis: Mapping

Cheat #4: Jamie's 30 Minute Meals!

Shared Data → Published Histories →

ESHG 1: Through BWA Mapping w/ RG →
Import History

One thing we aren't showing that we also ran is:

NGS: Picard → MergeSamFiles

Variant analysis: BAM Cleanup & Manipulation

Use only the good stuff!

NGS BAM Tools → Filter

Mapping Quality → **>=20**

Insert Filter → **isProperPair: Yes**

Insert Filter → **reference: chrM**

Variant analysis: BAM Cleanup & Manipulation

But not too much good stuff: **remove duplicates**

NGS Picard → MarkDuplicates

Remove Duplicates → **Yes**

Variant analysis: BAM Cleanup & Manipulation

Miscellaneous tidying

NGS Picard → CleanSam

Variant analysis: BAM Cleanup & Manipulation

Downsample it (hey, it's a workshop)

NGS Picard → Downsample SAM/BAM

~ 10%

Variant analysis

NGS Variant Analysis -> Naive Variant Caller

100	Minimum number of reads need to consider a REF/ALT
30	Minimum base quality
20	Minimum mapping quality
1	Ploidy
chrM	Restrict to regions
Yes	Report counts by strand

Produces a VCF file listing variants

Variant analysis: Only positions with variation

Lots of information in a VCF file

Can filter based on most of it.

NGS VCF Manipulation → VCFfilter

Get only positions with alternate allele frequency of 5%+

Specify filtering expression → -f "AF > 0.05"

Variant analysis: Annotation

NGS Variant Analysis -> Variant Annotator

1.0	Minor Allele Frequency threshold (in percent)
100	Coverage threshold (in reads per strand)
No	Do not filter sites or alleles
Yes	Output stranded base counts
Yes	Write header line

Outline

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Variant Analysis: Other options

Galaxy: Resources and Community

Done

Galaxy

Tools

NGS: Variant Analysis

BamLeftAlign indels in BAM datasets

FreeBayes - Bayesian genetic variant detector

Slice VCF to get data from selected regions

MAF boxplot Minor Allele Frequency Boxplot

Phylorelatives Relatedness of minor alelle sequences in NJ tree

CloudMap: in silico complementation Perform in silico complementation analysis on multiple tabular snpEff output files

CloudMap: Variant Discovery Mapping with WGS data Map a mutation using in silico bulk segregant linkage analysis using variants that are already present in the mutant strain of interest (rather than those introduced by a cross to a polymorphic strain).

CloudMap: Hawaiian Variant Mapping with WGS data Map a mutation by plotting recombination frequencies resulting from crossing to a highly polymorphic strain

CloudMap: EMS Variant Density

Galaxy

Tools

CloudMap: EMS Variant Density Mapping Map a mutation by linkage to regions of high mutation density using WGS data

Variant Annotator process variant counts

FASTA from allele counts Generate major and minor allele sequences from alleles table

Naive Variant Caller - tabulate variable sites from BAM datasets

Annotate a VCF dataset with custom filters

Varscan for variant detection

ANNOVAR Annotate VCF with functional information using ANNOVAR

NGS: VCF Manipulation

VCFfixup: Count the allele frequencies across alleles present in each record in the VCF file

VCFprimers: Extract flanking sequences for each VCF record

VCFcombine: Combine multiple VCF datasets

VCFbreakCreateMulti: Break multiple alleles into multiple records, or combine overallpoing alleles into a single record

Galaxy

Tools

VcfAllelicPrimitives: Split alleleic primitives (gaps or mismatches) into multiple VCF lines

VCFfilter: filter VCF data in a variety of attributes

VCFrandomSample: Randomly sample sites from VCF dataset

VCFgenotype-to-haplotype: Convert genotype-based phased alleles into haplotype alleles

VCFcommonSamples: Output records belonging to samples commong between two datasets

VCFselectsamples: Select samples from a VCF dataset

VCFleftAlign: Left-align indels and complex variants in VCF dataset

VCFtoTab-delimited: Convert VCF data into TAB-delimited format

VCFaddinfo: Adds info fields from the second dataset which are not present in the first dataset

VCFannotate: Intersect VCF records with BED annotations

VCF-VCFintersect: Intersect two VCF datasets

VCFannotateGenotypes:

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Tools

VCFannotateGenotypes: Annotate genotypes in a VCF dataset using genotypes from another VCF dataset

VCFcheck: Verify that the reference allele matches the reference genome

VCFsort: Sort VCF dataset by coordinate

VCFgenotypes: Convert numerical representation of genotypes to allelic

VCFhetHomAlleles: Count the number of heterozygotes and alleles, compute het/hom ratio

VCF-BEDintersect: Intersect VCF and BED datasets

VCFdistance: Calculate distance to the nearest variant

snpEff

CloudMap: Check snpEff Candidates Marks up a snpEff output file with matches to a gene candidate list.

SnpSift Filter Filter variants using arbitrary expressions

SnpEff Variant effect and annotation

BEDTools

EMBOSS

https://toolshed.g2.bx.psu.edu

Galaxy Tool Shed

Repositories Help User

2857 valid tools on Dec 16, 2014

Search

- Search for valid tools
- Search for workflows

Valid Galaxy Utilities

- Tools
- Custom datatypes
- Repository dependency definitions
- Tool d

All Repo

- Browse

Available

- Login

Repositories by Category

search repository name, description

Name	Description	Repositories
Assembly	Tools for working with assemblies	36
ChIP-seq	Tools for analyzing and manipulating ChIP-seq data.	10
Transcriptomics	Tools for use in the study of Transcriptomics.	16
Variant Analysis	Tools for single nucleotide polymorphism data such as WGA	164
Visualization	Tools for visualizing data	45
Data Source	Tools for retrieving data from external data sources	17
Fasta Manipulation	Tools for manipulating fasta data	42

Getting help on Variant Calling

- http://biostars.org/
- http://seqanswers.com/
- http://galaxyproject.org/search

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Done

Galaxy Community Resources

Galaxy Community Resources: Galaxy Biostar

Tens of thousands of users leads to a lot of questions.

Absolutely have to encourage community support.

Project traditionally used mailing list

Moved the user support list to Galaxy Biostar, an online forum, that uses the Biostar platform

https://biostar.usegalaxy.org/

Galaxy Community Resources: Mailing Lists

http://wiki.galaxyproject.org/MailingLists

Galaxy-Dev

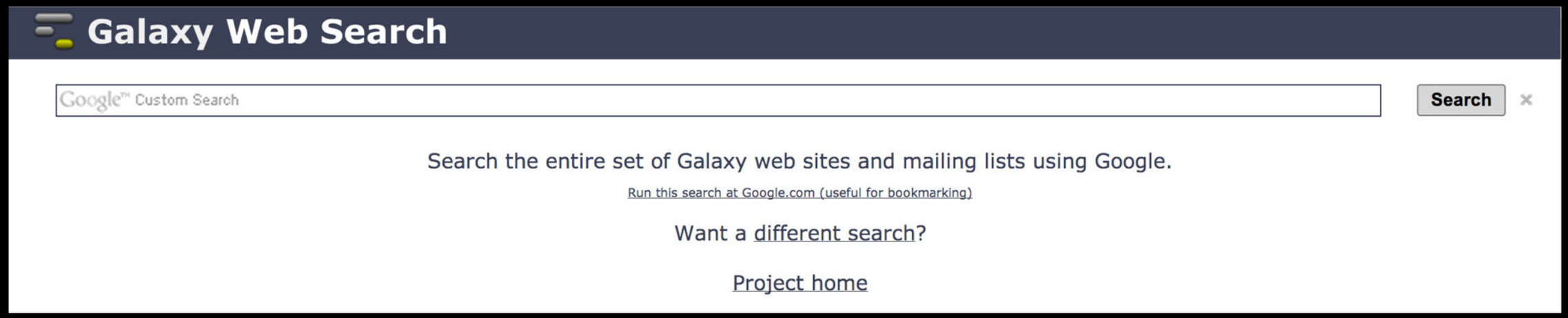
Questions about developing for and deploying Galaxy
High volume (3246 posts in 2014, 1000+ members)

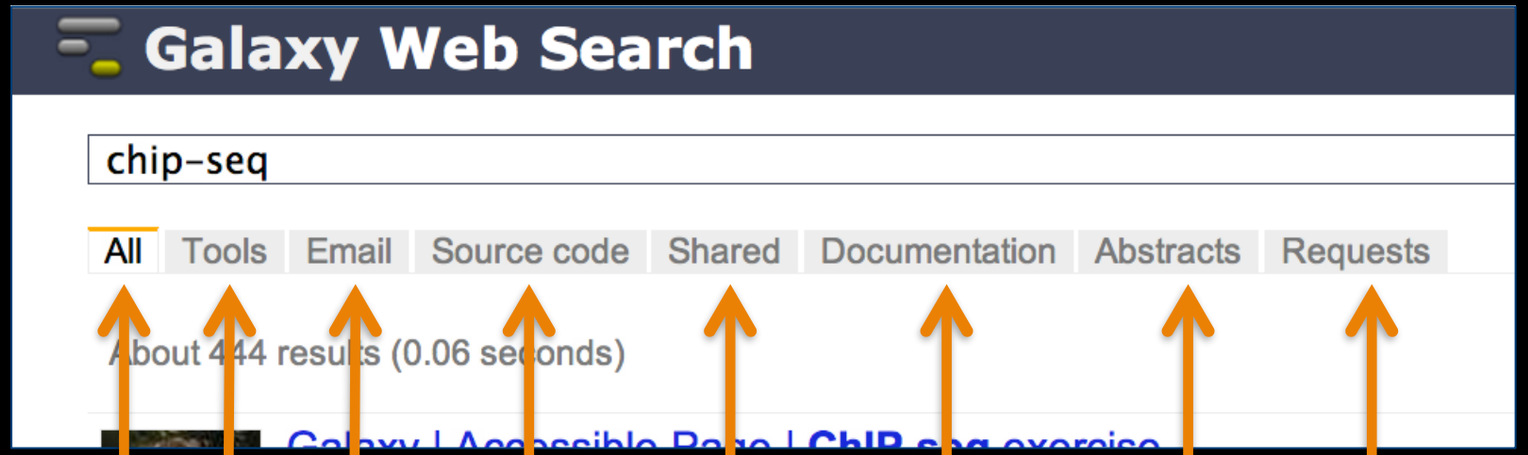
Galaxy-Announce

Project announcements, low volume, moderated
Low volume (34 posts in 2014, 4400+ members)

Also Galaxy-UK, -France, -Proteomics, -Training, ...

Unified Search: http://galaxyproject.org/search





Find

Everything on …

Tools for …

Email about …

Source code for …

Published Histories, Pages, Workflows, about …

Documentation on …

Papers using Galaxy for …

Related feature requests

http://wiki.galaxyproject.org

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

- **Accessible:** Users without programming experience can easily specify parameters and run tools and workflows.
- **Reproducible:** Galaxy captures information so that any user can repeat and understand a complete computational analysis.
- **Transparent:** Users share and publish analyses via the web and create Pages, interactive, web-based documents that describe a complete analysis.

This is the Galaxy Community Wiki. It describes all things Galaxy.

Use Galaxy

Galaxy's public web server usegalaxy.org makes analysis tools, genomic data, tutorial demonstrations, persistent workspaces, and publication services available to any scientist. Extensive user documentation applicable to any public or local Galaxy instance is available.

Deploy Galaxy

Galaxy is a free and open source project available to all. Local Galaxy servers can be set up by downloading the Galaxy application.

- Admin
- Cloud

Community & Project

Galaxy has a large and active user community and many ways to get involved.

- Community

Contribute

- **Users:** Share your histories, workflows, visualizations, data libraries, and Galaxy Pages, enabling others to use and learn from them.

Galaxy web search

Use Galaxy

Servers • Learn
Main • Choices
Share • Search

Communicate

Support • Biostar
Events • Mailing Lists
News • Twitter

Deploy Galaxy

Get Galaxy • Cloud
Admin • Tool Config
Tool Shed • Search

Contribute

Develop • Tools
Issues & Requests
Logs • Deployments
Teach

Galaxy Project

Home • About • Cite
Community
Big Picture

Events

News

Galaxy Wiki

DaveClements Settings Logout | Search: Titles Text

Events

Edit History Actions

Galaxy Event Horizon

Events with Galaxy-related content are listed here.

Also see the Galaxy Events Google Calendar for a listing of events and deadlines that are Galaxy Community. This is also available as an RSS feed.

If you know of any event that should be added to this page and/or to the Galaxy Event Calendar, send it to outreach@galaxyproject.org.

For events prior to this year, see the **Events Archive**.

Upcoming Events

Date	Topic/Event	Venue/Location
December 12	*Introduction to Galaxy Workshop*	Virginia State University, Petersburg, Virgin
December 16-19	*RNA-Seq and ChIP-Seq Analysis with Galaxy*	UC Davis, California, United States
		2015
January 10-14	*Galaxy for SNP and Variant Data Analysis*	Plant and Animal Genome XXIII (PAG2014), States
January 19-20	*NGS pipelines with Galaxy*	e-Infrastructures for Massively Parallel Sequ Sweden
February 9-13	*Analyse bioinformatique de séquences sous Galaxy*	Montpellier, France
February 16-18	*Accessible and Reproducible Large-Scale Analysis with Galaxy*	Genome and Transcriptome Analysis, pa Conference, San Francisco, Cali
	Large-Scale NGS data Analysis on Amazon Web Services Using Globus Genomic	Genomics & Sequencing Data Integration, of Molecular Medicine Tri-Conference, Sa States
	iReport: An Integrative "omics"	

News Items

Opening at McMaster University

The McArthur Lab in the McMaster University Department of Biochemistry & Biomedical Sciences is seeking a Systems Administrator / Information Technologist to help establish a new bioinformatics laboratory at McMaster, plus develop the next generation of the Comprehensive Antibiotic Resistance Database (CARD).

From the job announcement on EvolDir:

> The candidate will configure BLADE and other hardware for general bioinformatics analysis, development of a GIT version control system, **construction of an in house Galaxy server (usegalaxy.org)**, and development of a new interface, stand-alone tools, APIs, and algorithms for the CARD (based on Chado).

See the full announcement for details.

Posted to the Galaxy News on 2014-12-05

December 2014 Galaxy Newsletter

As always there's a lot going on in the Galaxy this month. "Like what?" you say. Well, read the dang December Galaxy Newsletter we say! Highlights include:

- Galaxy Day! In Paris! This Wednesday!
- Near Richmond, Virginia? There's a Galaxy Workshop at Virginia State U on December 12.
- GCC2015 needs sponsors!
- Other upcoming events on two continents
- **96 new papers**, including 6 highlighted papers, referencing, using, extending, and implementing Galaxy.
- Job openings at 7+ organizations
- A new mailing list: Galaxy-Training
- 15 new ToolShed repositories from 10 contributors
- And, 10 other juicy (well maybe not *juicy*, but certainly not *crunchy*) bits of news

Dave Clements and the *crisp* Galaxy Team

Posted to the Galaxy News on 2014-12-01

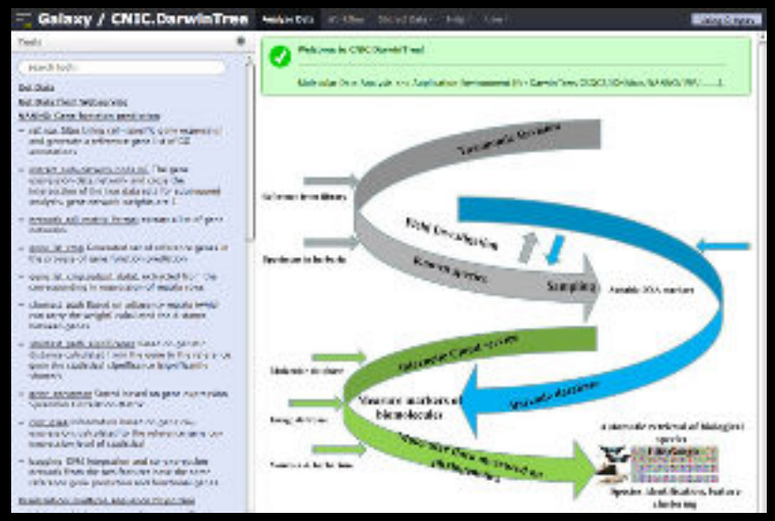
Bioinformaticians, Freiburg

Max Planck Institute of Immunobiology and Epigenetics in Freiburg, Germany has an opening for a Bioinformatician for an initial period of two years. The successful candidate will work at the interface between an in-house deep-sequencing facility (HiSeq-2500) and the various research groups at the institute. Main responsibilities include

- primary analysis of deep-sequencing data and quality controls

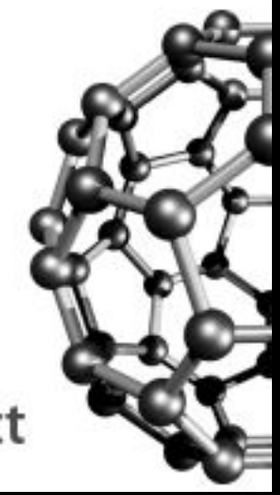
Cistrome

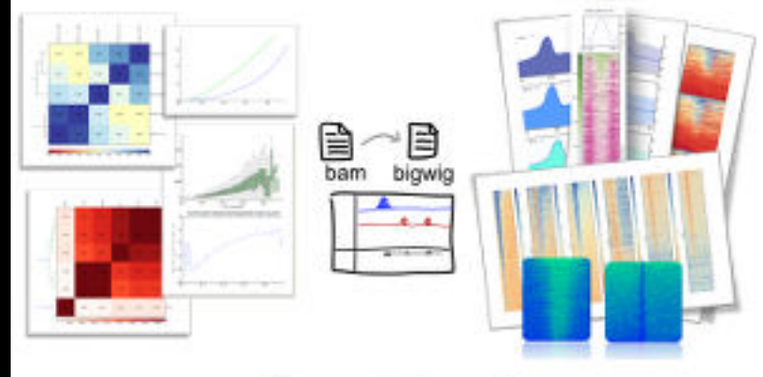
A Galaxy Server dedicated to ChIP-* analysis



ballaxy

Powered by the Biochemical Algorithms Library Project





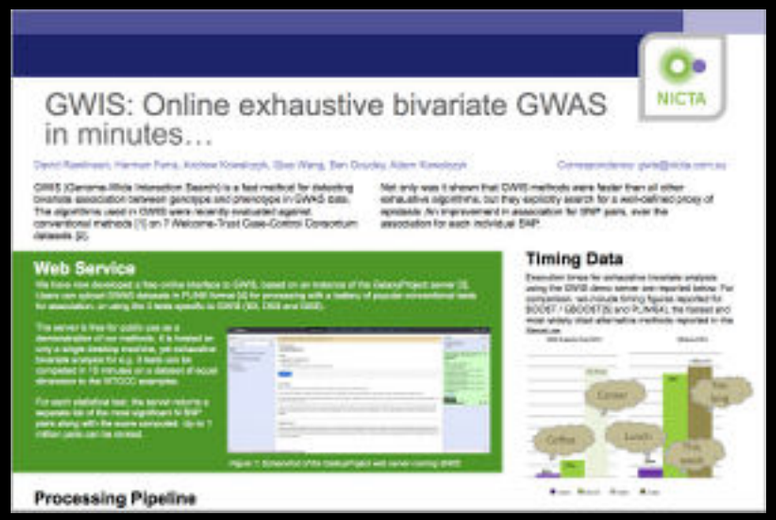
deepTools

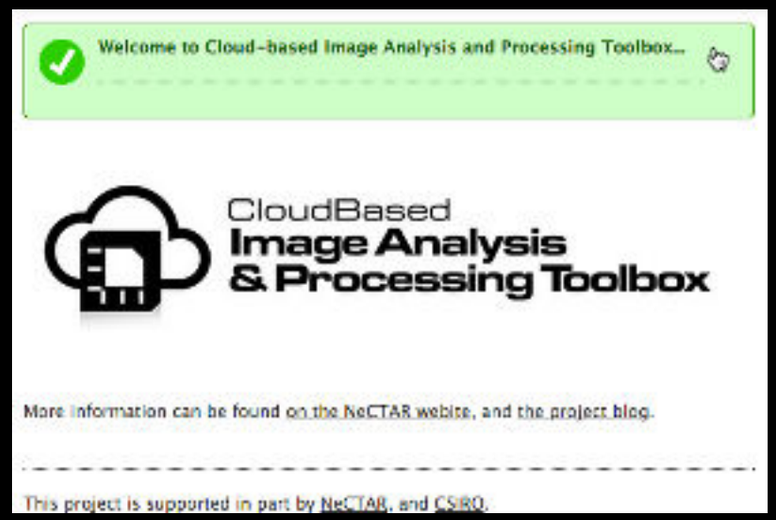
CoSSci

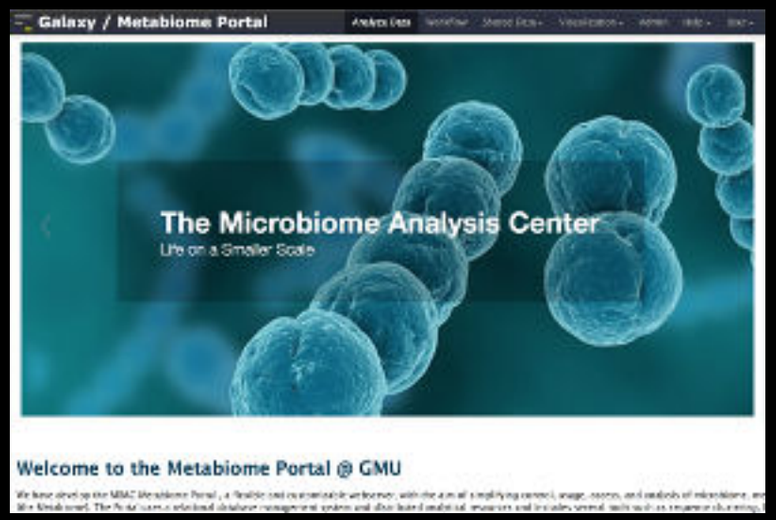
Galaxy for Complex Social Sciences

GENEONTOLOGY
Unifying Biology

galaxy.berkeleybop.org







bit.ly/gxyServers

Community can create, vote and comment on issues

HOME TOUR GOLD BUSINESS CLASS BLOG Trello Sign Up Log In

Want to subscribe, vote or comment on these cards? Sign up for free or learn more about Trello

Galaxy: Development

Public

Inbox

To add cards, use http://galaxyproject.org/trello
4 votes 2

To request reference genome, comment on this card.
1 vote 5 0/6

Toolshed installation fails silently
3 votes 1

Handle cluster job preemption
2 votes 1

Return code 271 causes traceback for PBS torque
1 vote 2

BUG: Tool shed repository export to capsule does not always capture all dependencies
1 vote 1

Remove manual_builds.txt from source control and replace with a .sample version
1 vote 1

Tool Requests

595: Add SAMTools "Sort"
4 votes 13

601: SAM-to-BAM tool enhancements
2 votes 1

Tools: Add tool to generate simulated reads to Main
3 votes 1

default max insert size of Bowtie2 should be increased
2 votes 5

307: A tool to produce a set of random intervals.
2 votes 2

Converter Tool: SAM to BAM enhancements
2 votes

New Tool: convert IUPAC chars to N
1 vote 7 1

Bug Reports

Usability: expanding datasets near the bottom of panel
CE

Bug: SICER on Main dependency issue
2 votes 20 3/5

Profile Annotations bad values when "select all"
1 vote 5

Filter pileup tool doesn't recognize pileup output data
1 vote 2

Bug: Odd Fetch Taxonomy tool behavior
1 vote 1

Strip message after pause jobs resumed
1 vote 1

Ideas

697: Workflow job control functions
10 votes 9

User Metrics and Analytics
3 votes 3 1/2
CE

Tuxedo RNA-seq tools: report command-line
2 votes 3

Tools: Incorporate key Cuffdiff output files for Cummerbund
2 votes 1 0/3

Moving objects between Galaxy instances, data federation, distributed storage, and data locality
2 votes

Workflow Editor: Provide explicit access to implicit datatype converter tools
1 vote

Pull Re

665: P issue

Custo
2 vo

Tools: Reque
3 vo

add rn downs
2 vo

Please wrapp mappi
2 vo

[galax libxml
1 vo

Pull Re manag

Menu

Members

CE NS G

Activity

Lance Parsons on Add or update wrappers for SamTools 1.0

I see that @peterjc has a wrapper for idxstats already and that it's listed on this card as "done" but I don't see it in the github repo. Will idxstats become part of this devteam collection or should I just start using the wrapper from @peterjc (Thanks Peter!)

today at 3:52 pm

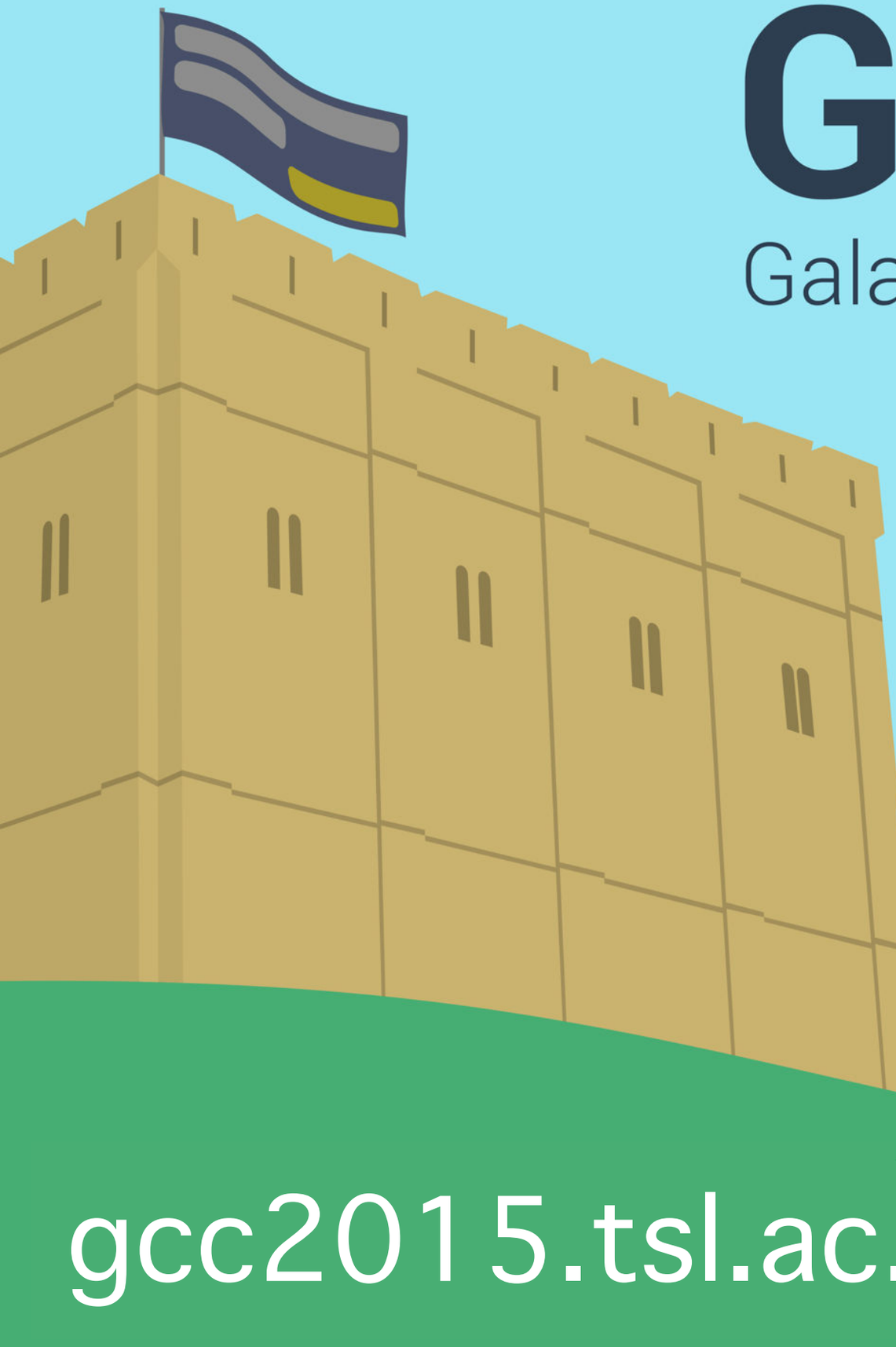
G **g2roboto** added Pull Request #606 - [STABLE] Escape instances of message passed in through kwd before pushing them back out to

http://bit.ly/gxytrello

GALAXY COMMUNITY CONFERENCE

BALTIMORE, MD | JUNE 30 - JULY 2, 2014

Slides, posters & videos now online
http://bit.ly/gcc2014



GCC 2015

Galaxy Community Conference

4 6-8th July 2015

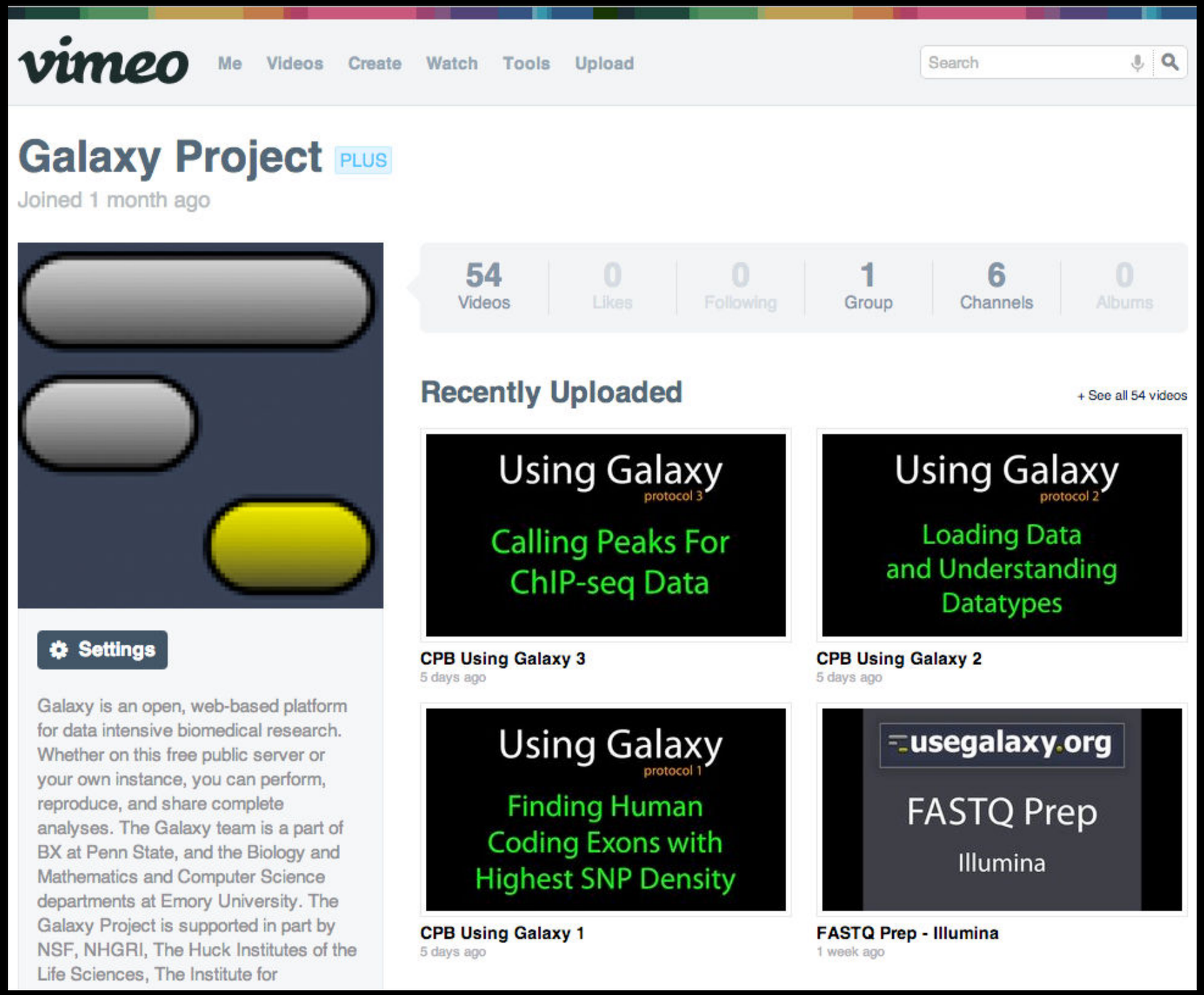
The Sainsbury Laboratory
Norwich, UK

gcc2015.tsl.ac.uk

GCUK IS LIVE!

We also support community organized efforts and events.

Galaxy Resources & Community: Videos



"How to" screencasts on using and deploying Galaxy

Talks from previous meetings.

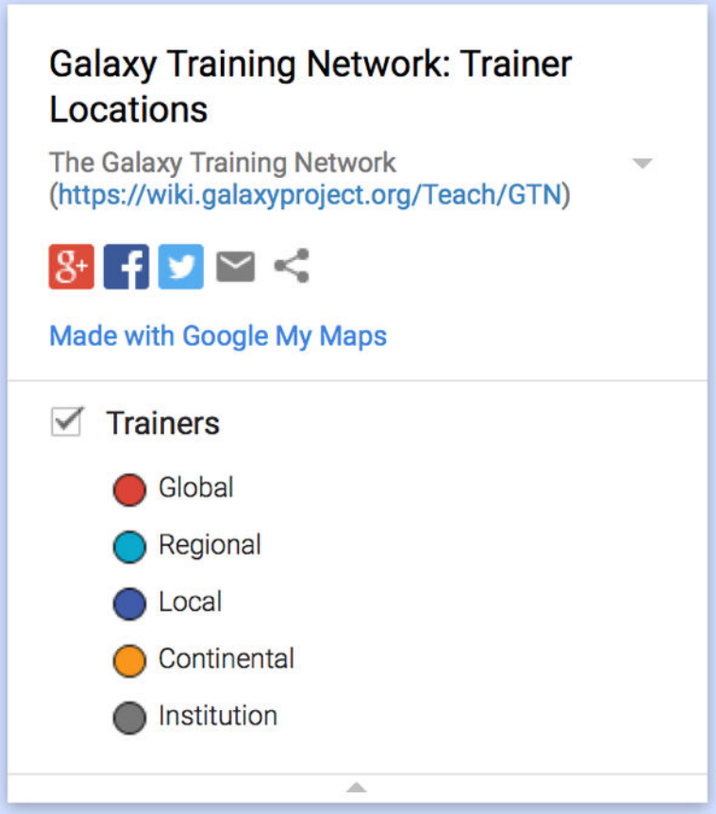
http://vimeo.com/galaxyproject

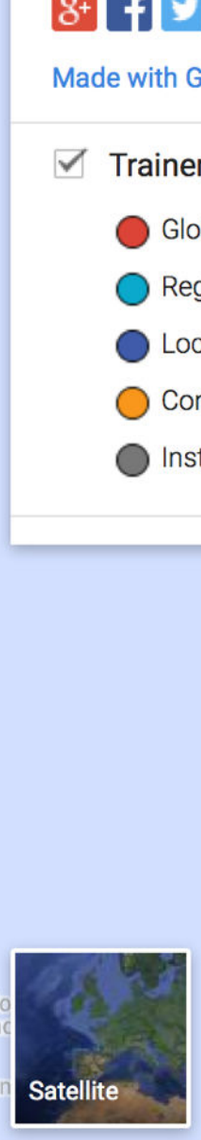
Galaxy Resources & Community: CiteULike Group

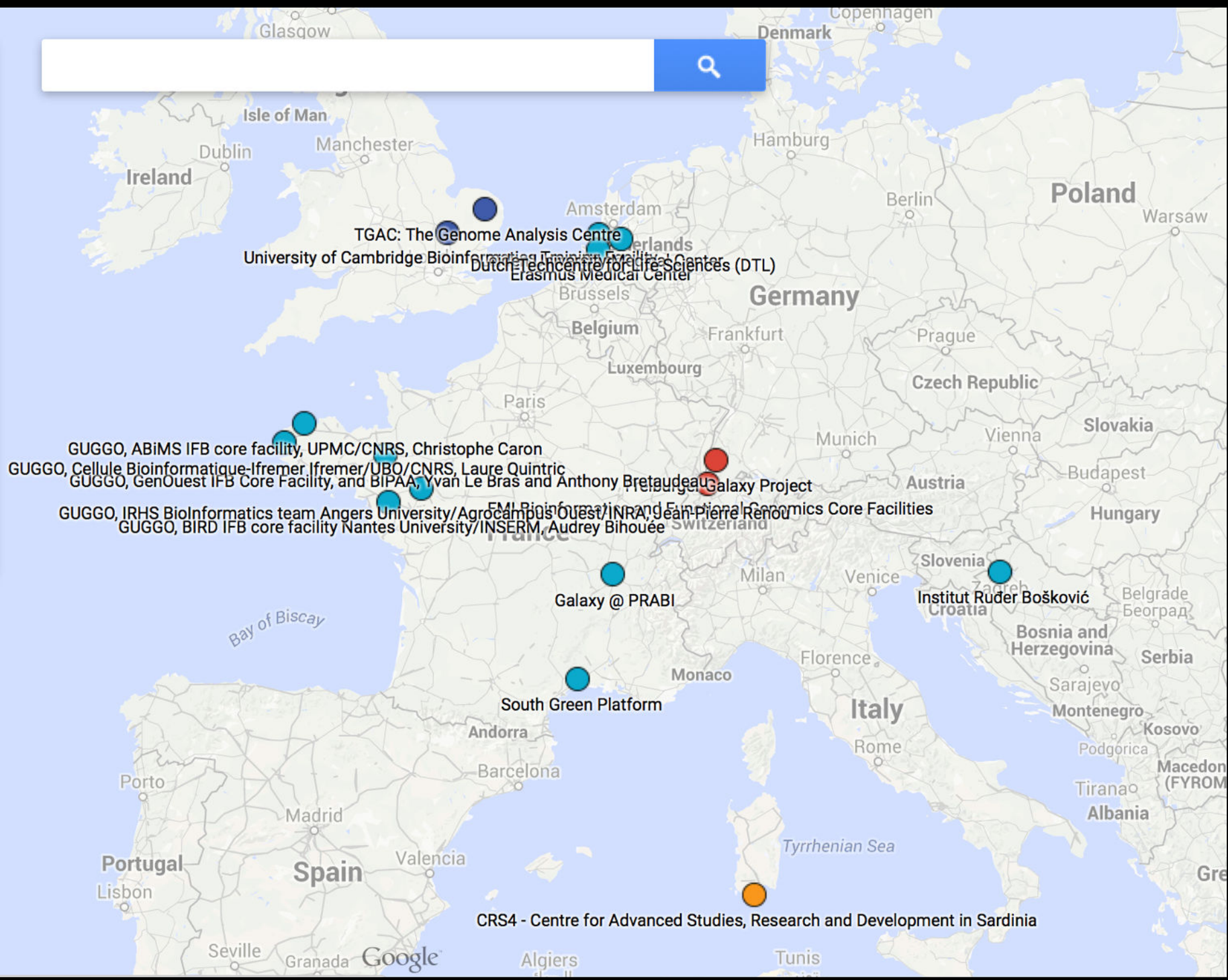
Now over 2300 papers

http://bit.ly/gxycul

Scaling Training







Galaxy Training Network launched In October.

bit.ly/gxygtn

Outline

Introduction: The Galactic Landscape

Variant Analysis: A worked example

Variant Analysis: Other options

Galaxy: Resources and Community

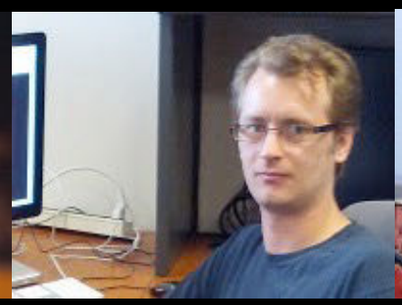
Done (almost)

The Galaxy Team

Enis Afgan

Dannon Baker

Dan Blankenberg



Dave Bouvier

Marten Cech

John Chilton

Dave Clements

Nate Coraor

Carl Eberhard

Jeremy Goecks

Sam Guerler

Jen Jackson

Ross Lazarus

Anton Nekrutenko

Nick Stoler

James Taylor

Nitesh Turaga

http://wiki.galaxyproject.org/GalaxyTeam

Galaxy is hiring post-docs and software engineers

Please help.

http://wiki.galaxyproject.org/GalaxyIsHiring

Also Thanks To

NIH National Institutes of Health

Brunhilde Wirth
Stephen
Carole

Outline

Introduction: The Galactic Landscape

Variant Analysis: A worked example

Variant Analysis: Other options

Galaxy: Resources and Community

Done

Slides at http://bit.ly/XXXX

Thanks

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