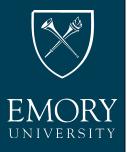


Understanding Cancer Genomes (and Transcriptomes!) using Galaxy Jeremy Goecks

html file (html

Department of Biology
Department of Math and Computer Science
Emory University



Roadmap

Galaxy

Analyzing Cancer Genomes and Transcriptomes

Vision

Galaxy is an open, Web-based platform for accessible, reproducible, and collaborative computational genomics

What is Galaxy?

GUI for high-throughput, high-performance genomics

- 1. get and integrate public, private data
- 2. analyze data and create workflows
- 3. visualization and visual analysis, sharing, publication

Customizable open-source software on various HPC resources

- public website http://usegalaxy.org
- local instance
- on the cloud

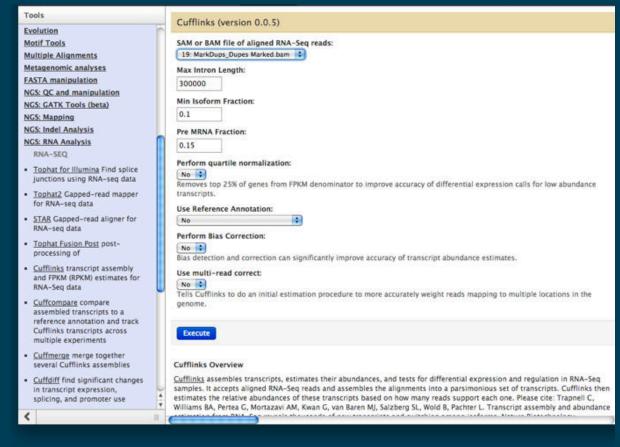
Galaxy Demo

Accessibility

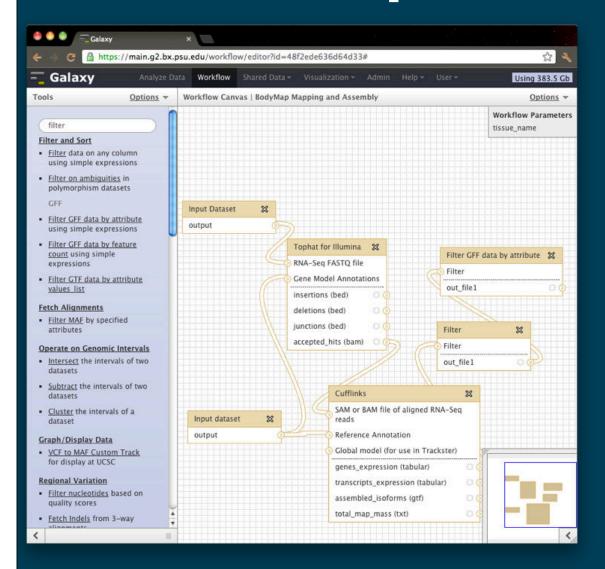
All tools looks the same

No command line or programming

Easy to chain tools together into larger analyses

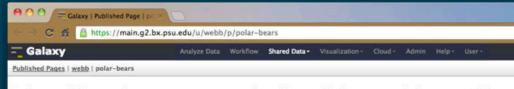


Reproducibility



Workflows enable reuse and provide precise reproducibility

Users can add tags and annotations for additional context



Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change

Webb Miller, Stephan C. Schuster, Andreanna J. Welch, Aakrosh Ratan, Oscar C. Bedoya-Reina, Fangging Zhao, Hie Lim Kim, Richard C. Burhans, Daniela I. Drautz, Nicola E. Wittekindt, Lynn P. Tomsho, Enrique Ibarra-Laclette, Luis Herrera-Estrella, Elizabeth Peacock, Sean Farley, George K. Sage, Karyn Rode, Martyn Obbard, Rafael Montiel, Lutz Bachmann, Ólafur Ingólfsson, Jon Aars, Thomas Mailund, Øystein Wiig, Sandra L. Talbot, and Charlotte Lindqvist

Summary of the paper

Polar bears (PBs) are superbly adapted to the extreme Arctic environment and have become emblematic of the threat to biodiversity from global climate change. Their divergence from the lower-latitude brown bear provides a textbook example of rapid evolution of distinct phenotypes. However, limited mitochondrial and nuclear DNA evidence conflicts in the timing of PB origin as well as placement of the species within versus sister to the brown bear lineage. We gathered extensive genomic sequence data from contemporary polar, brown, and American black bear samples, in addition to a 130,000- to 110,000-y old PB, to examine this problem from a genome-wide perspective. Nuclear DNA markers reflect a species tree consistent with expectation, showing polar and brown bears to be sister species. However, for the enigmatic brown bears native to Alaska's Alexander Archipelago, we estimate that not only their mitochondrial genome, but also 5-10% of their nuclear genome, is most closely related to PBs, indicating ancient admixture between the two species. Explicit admixture analyses are consistent with ancient splits among PBs, brown bears and black bears that were later followed by occasional admixture. We also provide paleodemographic estimates that suggest bear evolution has tracked key climate events, and that PB in particular experienced a prolonged and dramatic decline in its effective population size during the last ca. 500,000 years. We demonstrate that brown bears and PBs have had sufficiently independent evolutionary histories over the last 4-5 million years to leave imprints in the PB nuclear genome that likely are associated with ecological adaptation to the Arctic environment.

Datasets

Many of the analyses reported in the paper were based on the five datasets given here. (You can also find them under Shared Data -> Data Libraries -> Genome Diversity, then under bear and dog.)

The first consists of 12,023,192 dog-based "SNPs", i.e., positions in the dog genome where we detected two distinct nucleotides in the corresponding bear locations (among the our three bear species, polar bear, brown bear, and American black bear). Each row in the table corresponds to a SNP, and has 124 entries.

Galaxy Dataset | bear SNPs

H08

The "bear assembly SNPs" table contains 13,038,705 putative SNPs that were identified using a de novo assembly of the polar bear genome (rather than the dog assembly). Each row of the table corresponds to a SNP, and has 117 columns.

Galaxy Dataset | bear assembly SNPs

HO#

The "bear mitochondrial SNPs" table contains 1,698 positions where not all 28 individuals had the same nucleotide. Each row represents one of these SNPs, and has 31 columns.

Galaxy Dataset | bear mitochondrial SNPs

The "bear SAPs" table contains 79,501 variant position in putative protein-coding regions, both synonymous and non-synonymous changes, Each row has 11

Galaxy Dataset | bear SAPs

One of the workflows (bear sweep table) uses a streamlined file with the locations of 19,014 dog genes (basically, each one is the longest of a set of overlapping splice variants). Each gene corresponds to a row of the table, which has 5 columns.

Galaxy Dataset | dog genes

Workflows

columns.

This page presents three "workflows" that produce results presented in the polar-bear paper. Almost all of the commands that they use are from the "Genome Diversity" tool set. (See the left panel under "Analyze Data".)

The first workflow generates the data for Figure 4A of the paper. (Those data were used to produce a more attractive PCA plot that includes other information.) The workflow needs to be applied to the "bear SNPs" data set as follows: (1) Under "Analyze Data" (in the black bar) create an empty history. (2) Under "Shared Data" -> "Published Pages", view this page. (3) Import the "bear SNPs" data set ("+" in the green circle near the right of the green bar), then click on "return to the previous page". (4) Import the "Bear PCA" workflow, and click on "start using this workflow". (5) You will be taken to your Workflow page, which will have a workflow called nported bear PCA"; click on it and select "run". (6) You will be taken to a history that includes the bear SNPs and the PCA workflow; scroll to the bottom of the workflow (middle panel) and press "Run workflow". (7) After the commands run (which takes a couple of minutes), click on the "eye" for the PCA command and look at the three Outputs. [Currently, the PCA workflow exposes an internal error- a so-called "race condition" -- In Galaxy, which may cause the PCA command to fail. If that happens, you can re-run the PCA (not the entire workflow) by clicking on the line that says something like "7: PCA on data 6", clicking on the blue re-run button, and clicking on "Execute". You also may need to give Galaxy a minute after the workflow finishes to put the output files in the correct places.]

Galaxy Workflow | bear PCA

The second workflow produces the admixture map for the two ABC bears, showing the genomic intervals (relative to the dog assembly) where one or both of an ABC bear's autosomes is (are) more like the consensus of the polar-bear genome than like the genome of the non-ABC brown bear (called "GRZ" in the paper). The figure produced by running the workflow is a small improvement over Figure S12 of the supplement (which has one chromosome shown in Figure 48 of the main paper). The new figure indicates the 3Mb interval on the left end of each dog chromosome, which are treated as heterchromatin in the dog assembly (i.e, containing only 3 million copies of the letter "N"). When you run the workflow, the last command produces two history items. The "eye" in the first one shows a text file giving coordinates of the genomic intervals where chromosomes look most like a particular group of individuals. The second "eye" leads you to the graphical picture and additional information.

Galaxy Workflow | bear admixture map

The third workflow produces a table of the 58 highest-scoring genomic intervals (relative to the dog assembly) showing signs of a "selective sweep" in polar bears, i.e., where an allele having a selective advantage increased in frequency in the population and brought along with it the neighboring alleles. The table appeared as Table S8 in the Supplement, and one interval is shown in Figure 7 of the main paper. To run the workflow you will need to place both the "bear SNP" file and the dog genes" file in your history. (Make sure before you press "Run workflow" that the workflow's inputs are connected to the proper files.) When the workflow has run, you can click on the "eye" for the last command to see the table.

Galaxy Workflow | bear sweep table



Communication and Reuse





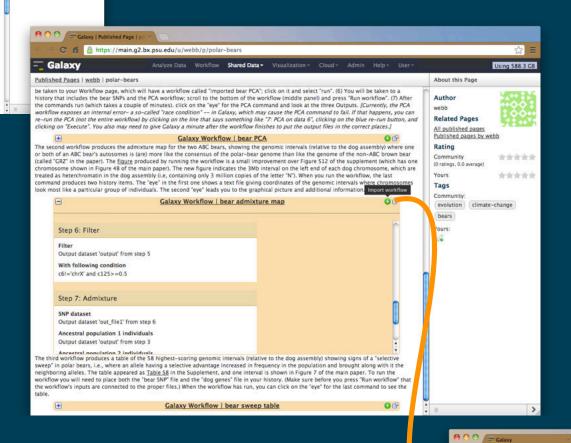
hese SNPs, and has 31 columns.

Galaxy Dataset | bear mitochondrial SNPs

The "bear SAPs" table contains 79,501 variant position in putative protein-coding regions, both synonymous and non-synonymous changes. Each ow has 11 columns.

Galaxy Dataset | bear SAPs

One of the workflows (bear sweep table) uses a streamlined file with the locations of 19,014 dog genes (basically, each one is the longest of a set of overlapping splice variants). Each gene corresponds to a row of the table, which has 5 columns.



C & https://main.g2.bx.psu.edu/workflow/imp?id=b7b9

Workflow "bear admixture map" has been imported. You can start using this workflow or return to the previous page.

Galaxy

Roadmap

Galaxy

Analyzing Cancer Genomes and Transcriptomes

Cancer Genomics

The New Hork Times

March 26, 2013

New Prostate Cancer Tests Could Reduce False Alarms

By ANDREW POLLACK

Sophisticated new prostate cancer tests are coming to market that might supplement the unreliable P.S.A. test, potentially saving tens of thousands of men each year from unnecessary biopsies, operations and radiation treatments.

Some of the tests are aimed at reducing the false alarms, and accompanying anxiety, caused by elevated P.S.A. readings. Others, intended for use after a definitive diagnosis, examine the genetic workings of the cancer to distinguish dangerous tumors that need treatment from slow-growing ones that might be left alone.

The New York Times

April 21, 2013

Cancer Centers Racing to Map Patients' Genes

By ANEMONA HARTOCOLLIS

The promise of whole genome sequencing can be seen in trials like one for bladder cancer at Memorial, where the effects of a drug normally used for breast cancer were disappointing in all but one of about 40 patients, whose tumor went away, Dr. Baselga said. Investigators sequenced the patient's whole genome. "The patient had a mutation in one gene that was right on the same pathway as the therapy," Dr. Baselga said. "And that explained why this worked."

Using Galaxy for Analysis of Cancer Genomes/Transcriptomes

New tools

complement existing transcriptome analysis tools

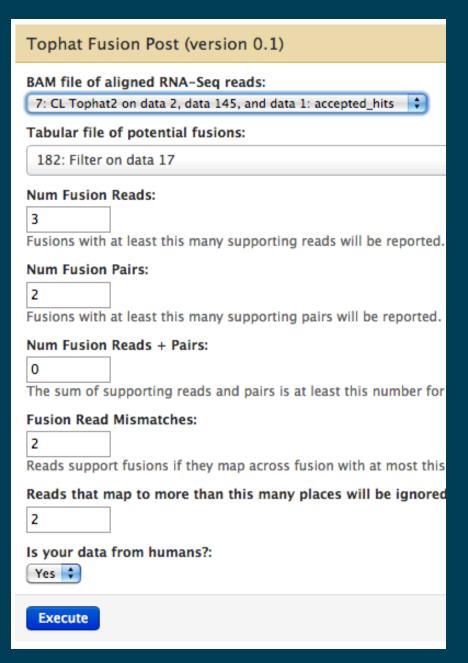
New workflows

workflows are understandable and extendable

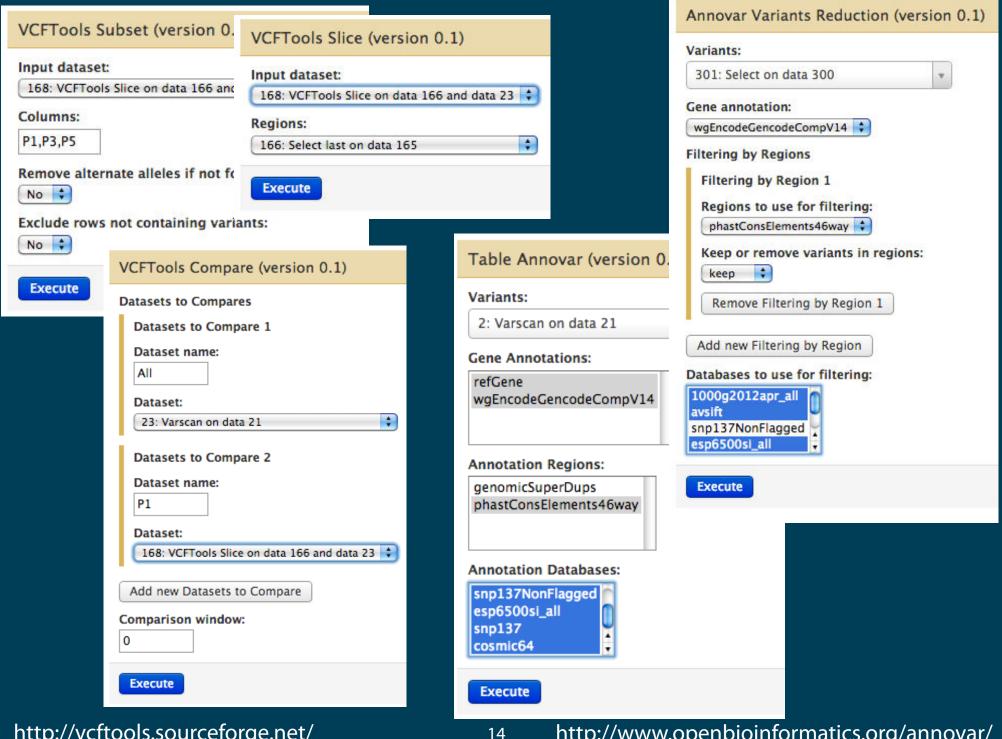
New visual analysis applications

visualize and call variants in a Web browser

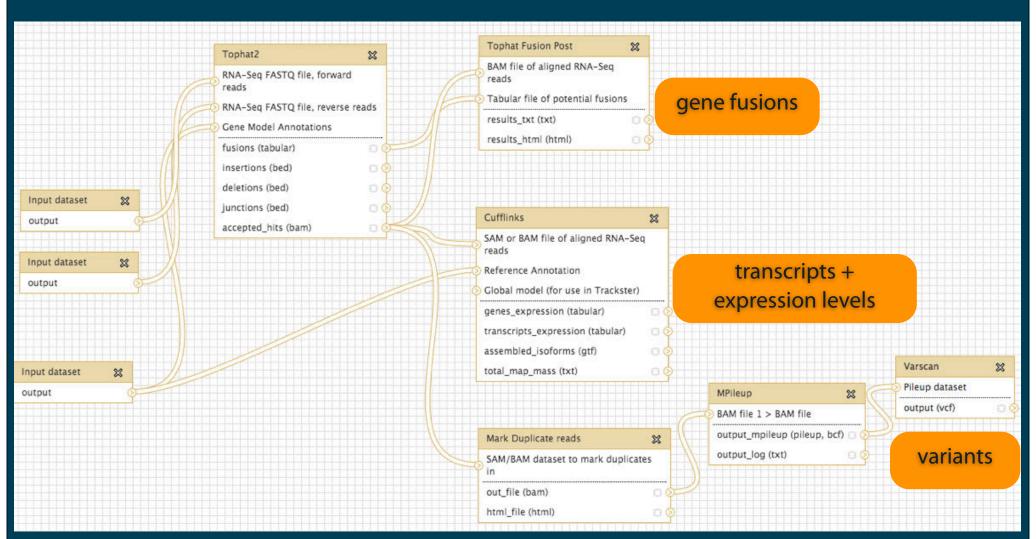
Varscan (version 0.1)
Pileup dataset: 21: MPileup on data 3, data 6, and others
Analysis type: single nucleotide variation 💠
Minimum read depth:
8 Minimum depth at a position to make a call
Minimum supporting reads:
Minimum supporting reads at a position to make a call
Minimum base quality at a position to count a read:
Minimum variant allele frequency threshold:
0.01
Minimum frequency to call homozygote: 0.75
p-value threshold for calling variants:
Ignore variants with >90% support on one strand:
no \$ sample_names:
Separate sample names by comma; leave blank to use default
Execute



http://tophat.cbcb.umd.edu/fusion_index.html



Single Sample Transcriptome Analysis

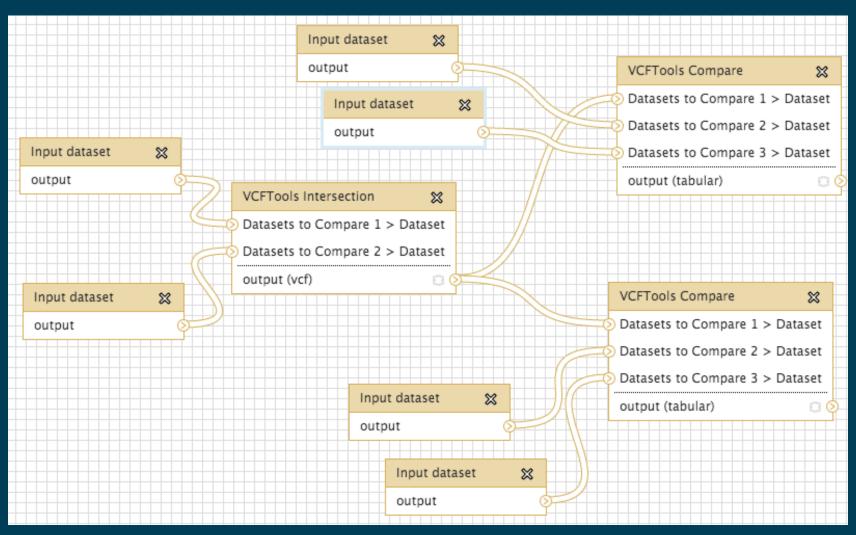


Aggregate Analysis

1. Differential expression with Cuffdiff

2. Join variants with MPileup

Comparing Called Variants with Public Datasets



Patient Mutations vs.



http://www.broadinstitute.org/ccle/home

	P1	P2	Р3	P4	P5	P6	CL
OM MIA (4)	0	1	1	0	0	0	4
OM PC (11)	0	1	1	0	0	0	4
OM ALL (114)	0	3	2	1	2	1	4
HP MIA (84)	6	6	5	5	4	4	19
HP PC (1769)	21	29	23	14	29	15	49
HP ALL (64,669)	247	357	332	214	280	233	263

OM = OncoMap, HP = hybrid capture with probes

Patient Mutations vs.



http://www.broadinstitute.org/ccle/home

	P1	P2	Р3	P4	P5	P6	CL
OM MIA (4)	0	1	1	0	0	0	4
OM PC (11)	0	1	1	0	0	0	4
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HP MIA (84)	6	6	5	5	4	4	19
HP PC (1769)	21	29	23	14	29	15	49
HP ALL (64,669)	247	357	332	214	280	233	263

Cell line does not appear very similar to tumors

OM = OncoMap, HP = hybrid capture with probes

Patient Mutations vs.

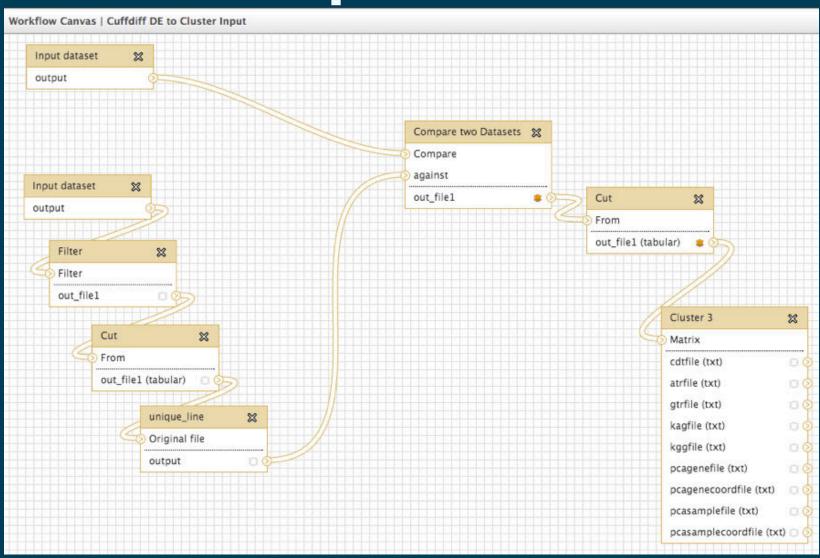


http://www.broadinstitute.org/ccle/home

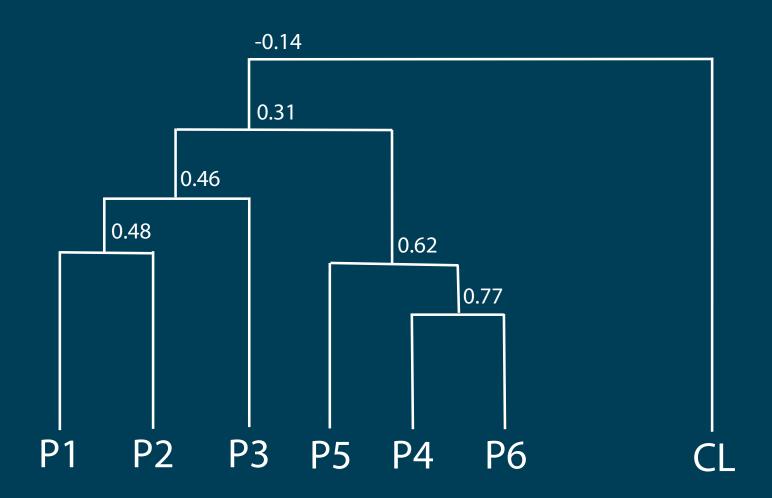
	P1	P2	Р3	P4	P5	P6	CL
OM MIA (4)	0	1	1	0	0	0	4
OM PC (11)	0	1	1	0	0	0	4
OM ALL (114)	0	3	2	1	2	1	4
HP MIA (84)	6	6	5	5	4	4	19
HP PC (1769)	21	29	23	14	29	15	49
HP ALL (64,669)	247	357	332	214	280	233	263
Tumor %	90%	90%	100%	0%?	60%	40%	

OM = OncoMap, HP = hybrid capture with probes

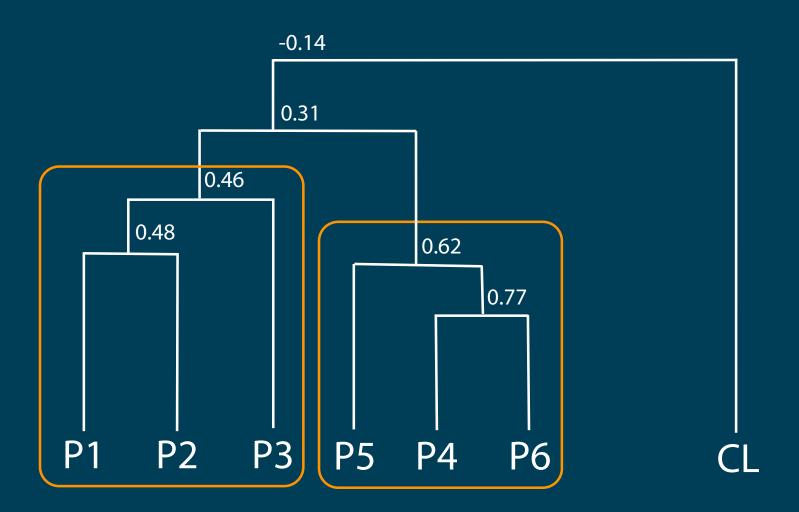
Clustering via Differential Expression



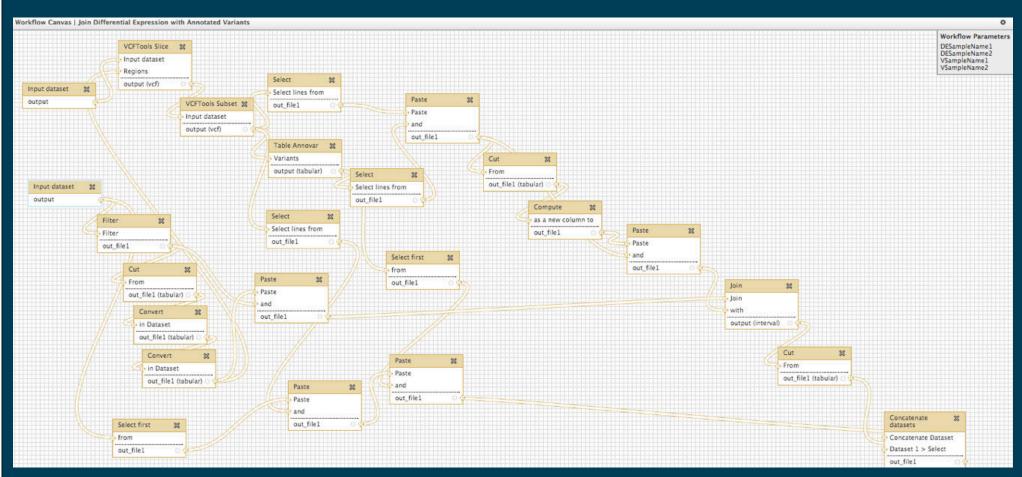
Gene Expression Clustering



Gene Expression Clustering



Variants + Gene Expression + Annotation



Finds and annotates variants in differentially-expressed genes or isoforms

Annotated Variants

1852 genes display differential expression (DE), ~5200 deleterious variants in DE genes

Have identified DE + mutations in known pancreatic cancer genes (e.g. REG1A)

step toward eQTL and drug targets

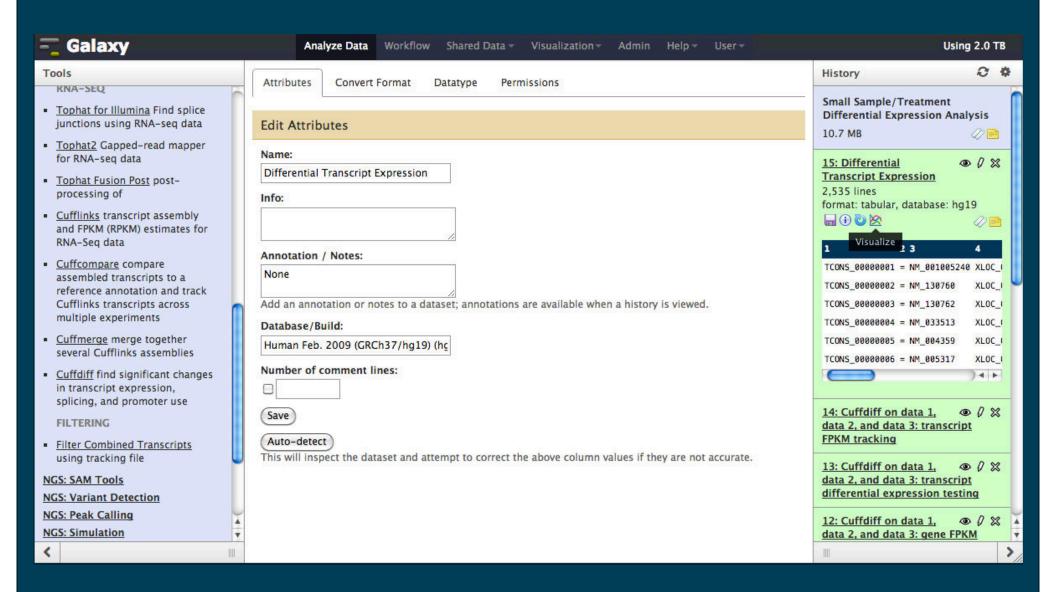
Galaxy Visualization and Visual Analysis

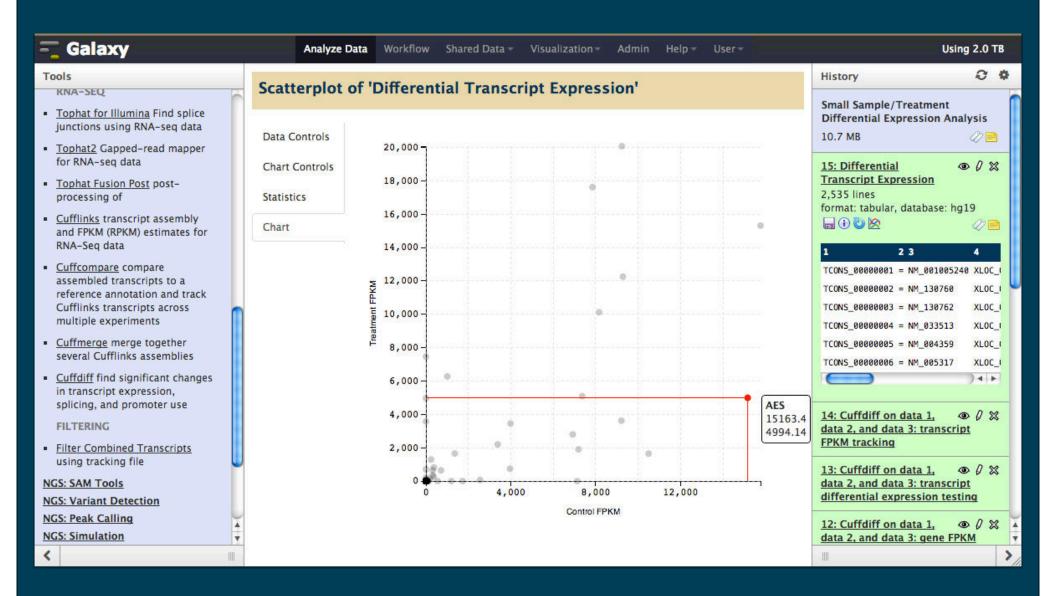
Visualization/visual analysis in Web browser

no software or data downloads

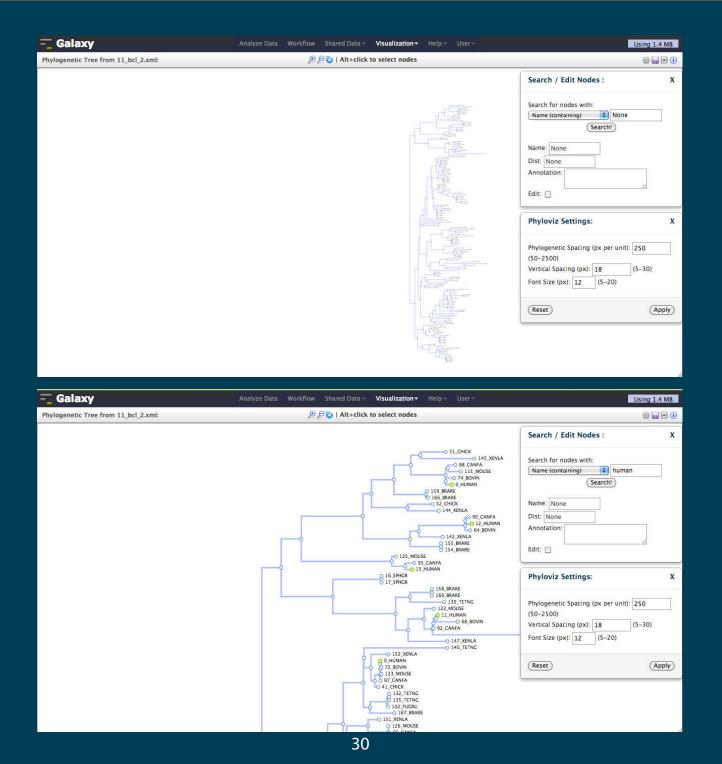
Scales to very large datasets

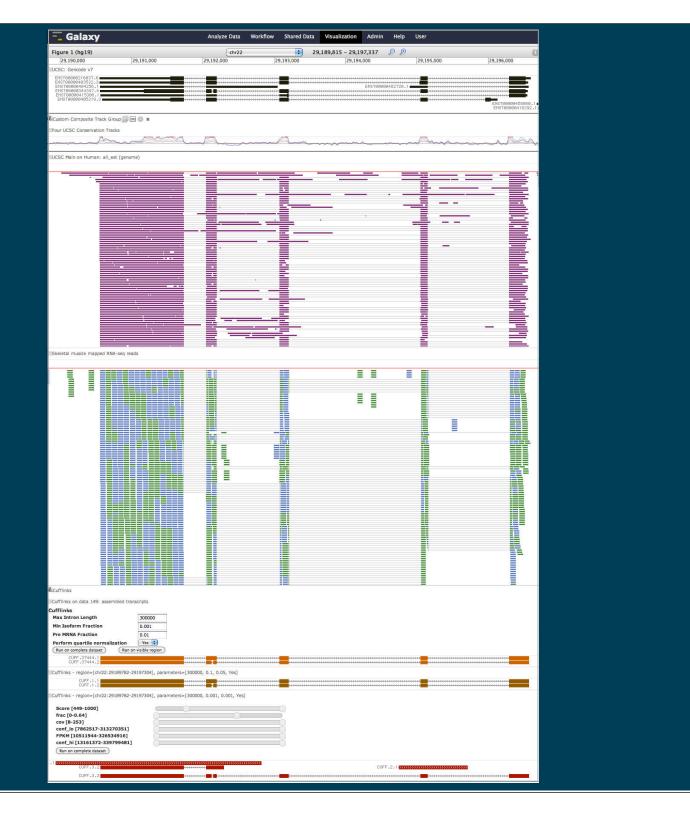
Can share and publish fully-functional visualizations











Visual Analysis Demo

Real-time Visual Analysis

Interactive use of production tool to call and visualize variants for multiple patients using parameter sweeps

Conclusions

New tools, workflows, and visual analysis tools for analyzing sequence data from cancer

ETA is October 2013, but can be set up locally now if motivated

Galaxy



Enis Afgan IRB



Guru Ananda Penn State



Dannon Baker Emory



Dan Blankenberg Penn State



Dave Bouvier Penn State



Dave Clements Emory



Nate Coraor Penn State



Carl Eberhard Emory



Jeremy Goecks Emory



Sam Guerler Emory



Jennifer Hillman Jackson Penn State



Greg von Kuster Penn State



Ross Lazarus BakerIDI



Anton Nekrutenko Penn State



James Taylor Emory



EMORY

WINSHIP CANCER **INSTITUTE**

A Cancer Center Designated by the National Cancer Institute



Mike Rossi







National Human Genome Research Institute National Institutes of Health



Thanks! And More:



http://galaxyproject.org http://usegalaxy.org

http://bitbucket.org/galaxy/galaxy-central http://wiki.galaxyproject.org

jeremy.goecks@emory.edu



1 Integrated Visualization & Computing Workshop

Tuesday, 14:10-16:05

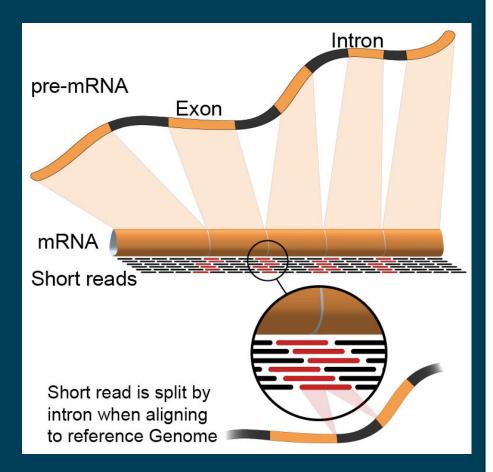
Preliminary Data

6 patients, whole transcriptome sequencing (RNA-seq) of primary tumor

mixed populations!

MiaPaCa2 cell line, whole transcriptome sequencing

Total sequencing data: 50 GB



http://en.wikipedia.org/wiki/RNA-Seq