CloudMap | Cloud-based Pipeline for Analysis of Mutant Genome Sequences

This user guide serves as a simplified, graphic version of the CloudMap paper for application-oriented end-users. For more details, please see the CloudMap paper. Video versions of these user guides and updates to the pipeline are available at the CloudMap website at: http://usegalaxy.org/cloudmap.

Helpful Galaxy screencasts are available at: http://wiki.g2.bx.psu.edu/Learn/Screencasts

Currently, all of the workflows (with the exception of ***EMS Density Mapping***) should work for any species as long as users provide the appropriate genome reference file (Fasta) where required. Instructions for configuring multi-species support for the ***Hawaiian Variant Mapping with WGS Data*** tool is provided in the ***Analyze Your Own Data Using CloudMap Workflows*** section of this user guide.

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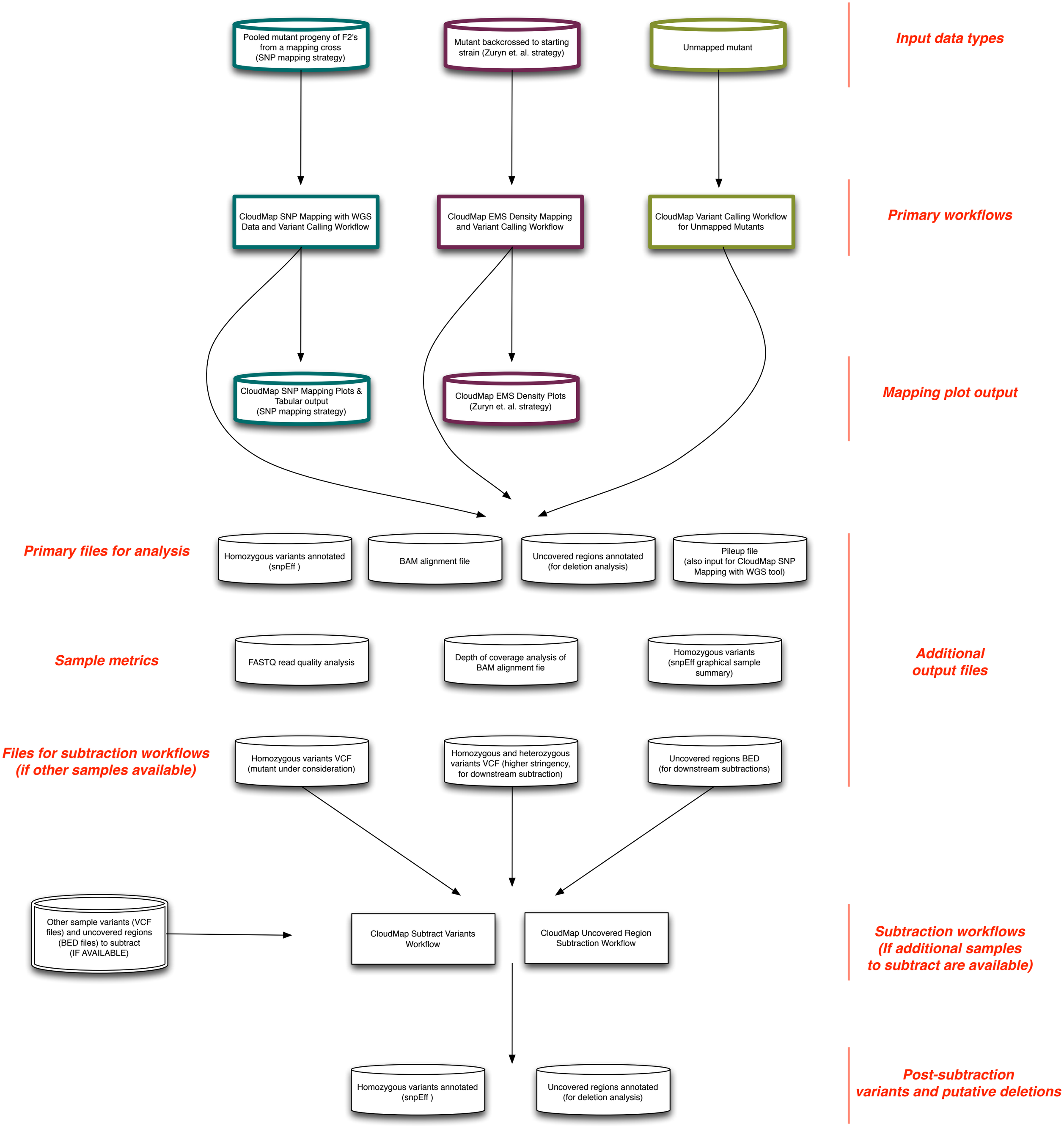
CONTENTS:


Input data types
Pooled mutant progeny of F2's from a mapping cross (SNP mapping strategy)
Mutant backcrossed to starting strain (Zuryn et. al. strategy)
Unmapped mutant
Primary workflows
CloudMap SNP Mapping with WGS Data and Variant Calling Workflow
CloudMap EMS Density Mapping and Variant Calling Workflow
CloudMap Variant Calling Workflow for Unmapped Mutants
Mapping plot output
CloudMap SNP Mapping Plots & Tabular output (SNP mapping strategy)
CloudMap EMS Density Plots (Zuryn et. al. strategy)
Primary files for analysis
Homozygous variants annotated (snpEff)
BAM alignment file
Uncovered regions annotated (for deletion analysis)
Pileup file (also input for CloudMap SNP Mapping with WGS tool)
Sample metrics
FASTQ read quality analysis
Depth of coverage analysis of BAM alignment fie
Homozygous variants (snpEff graphical sample summary)
Additional output files
Files for subtraction workflows (if other samples available)
Homozygous variants VCF (mutant under consideration)
Homozygous and heterozygous variants VCF (higher stringency, for downstream subtraction)
Uncovered regions BED (for downstream subtractions)
Other sample variants (VCF files) and uncovered regions (BED files) to subtract (IF AVAILABLE)
CloudMap Subtract Variants Workflow
CloudMap Uncovered Region Subtraction Workflow
Subtraction workflows (If additional samples to subtract are available)
Homozygous variants annotated (snpEff)
Uncovered regions annotated (for deletion analysis)
Post-subtraction variants and putative deletions

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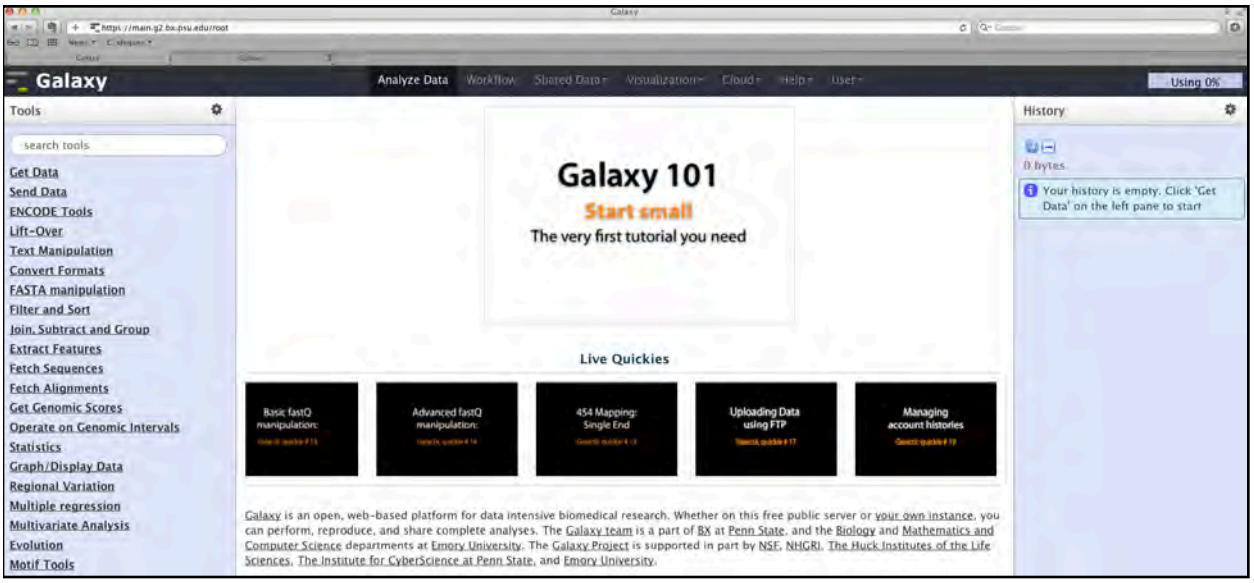
CloudMap Hawaiian Variant Mapping with WGS Data and Variant Calling Workflow (using *ot266* Proof of Principle example from the CloudMap paper). A video version of this user guide is available at: http://usegalaxy.org/cloudmap.

The *ot266* FASTQ file used in this example represents sequencing data from a specific kind of experiment: the *ot266* mutant has been crossed to a mapping strain (CB4856, "Hawaiian") and pooled F2 mutant progeny have been sequenced. This workflow uses single-end FASTQ data but it can be adapted to use paired-end data (see the ***Analyzing Your Own Data*** section of this user guide).

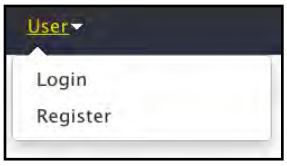
The aim in this user guide is to walk readers through Galaxy-based analysis of the *ot266* mutant using predefined CloudMap workflows which sequentially execute all of the steps required for common mutant analysis functions. This same workflow can be used for analysis of any mutant (from any species) that has been crossed to a mapping strain for which variant information is available.

These workflows provide default function parameters, ensuring that users follow best practices, and allow for automated execution of sequential operations. We provide these workflows as helpful guides, but experienced users may execute functions in any meaningful order they please and may also create and share their own workflows to take advantage of the automation feature. More CloudMap documentation is available at http://usegalaxy.org/cloudmap.

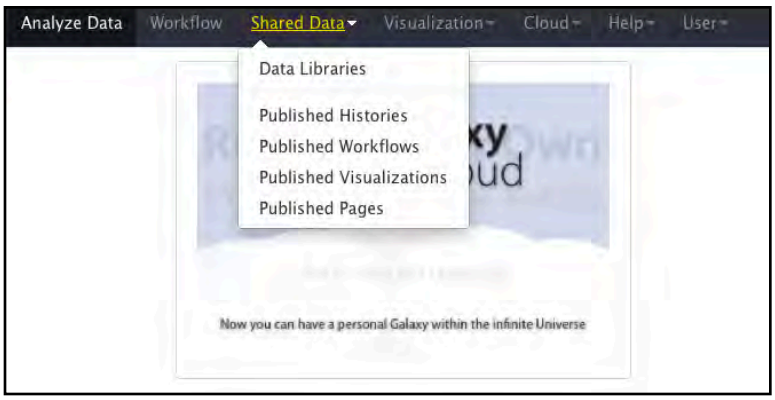
1) Navigate to http://usegalaxy.org (URL will resolve to something like https://main.g2.bx.psu.edu)



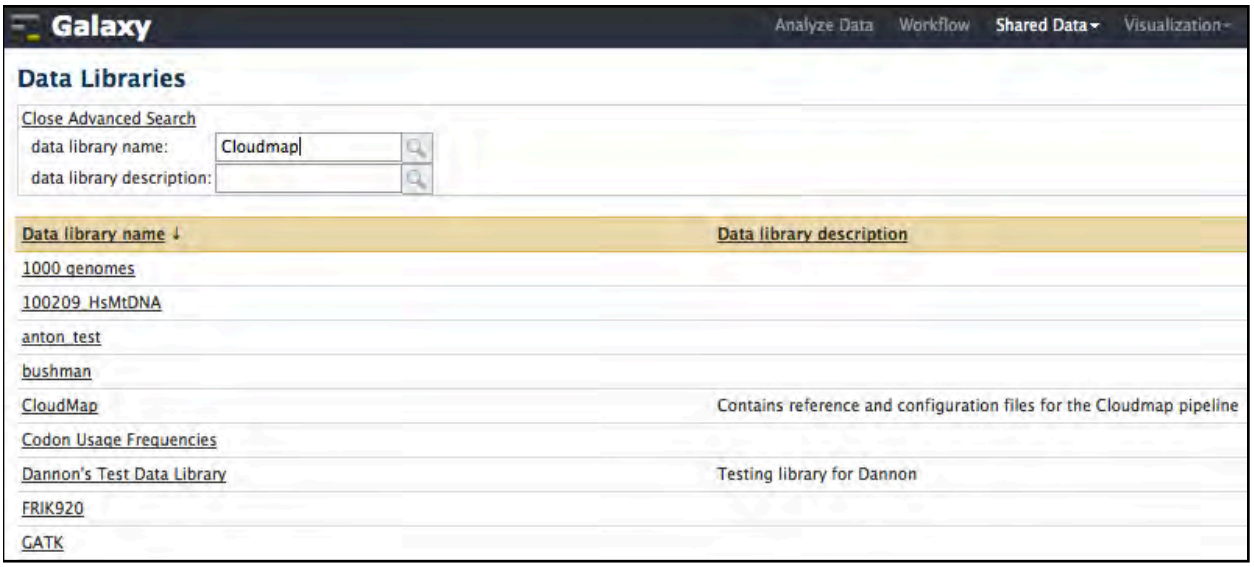
2) Register for an account or login if you already have an account:



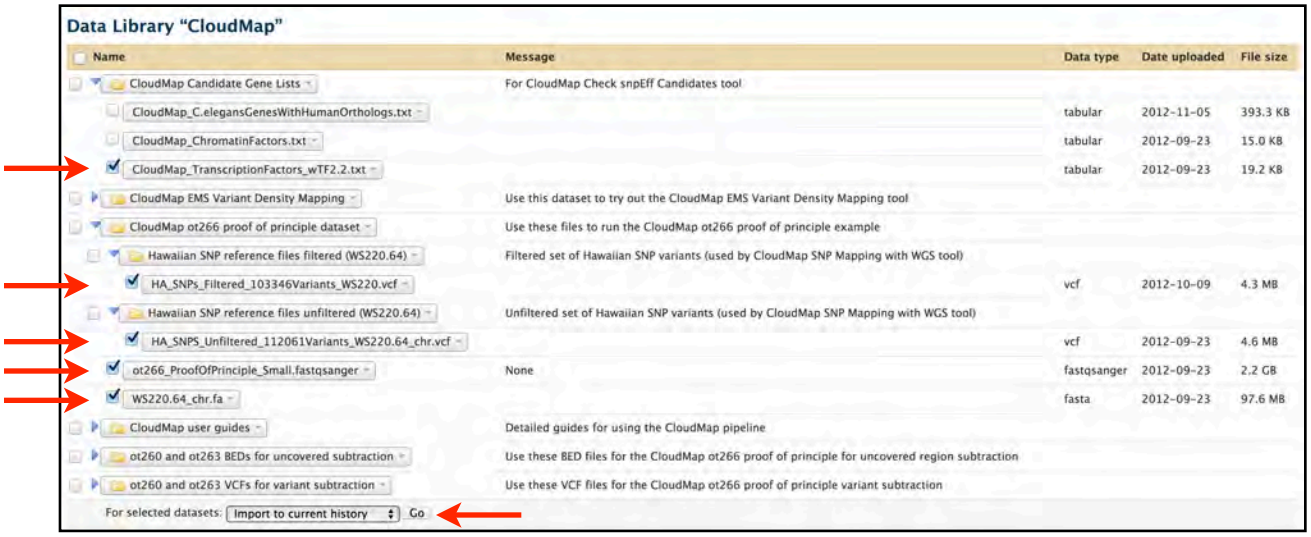
3) Once you are logged in using your email address, click on the ***Shared Data*** link at the top of the page:



4) Click on ***Data Libraries*** and search for the CloudMap data library:



5) Click on the ***CloudMap*** library and select the 5 data files below for the *ot266* example. Then click "Go" to import these files into your history.

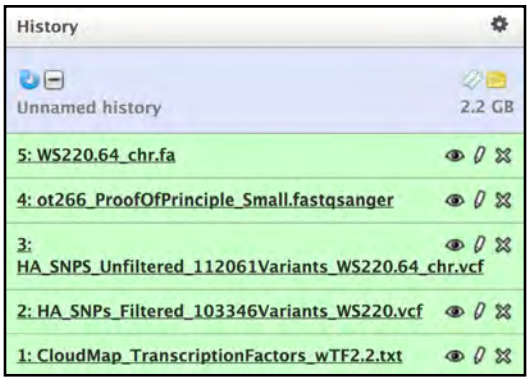


The filtered "HA_SNPS" file is used to generate SNP mapping plots (details in **Table S1** of the CloudMap paper). The unfiltered "HA_SNPs" VCF is used for variant subtraction as shown in **Fig.8**. of the CloudMap paper.

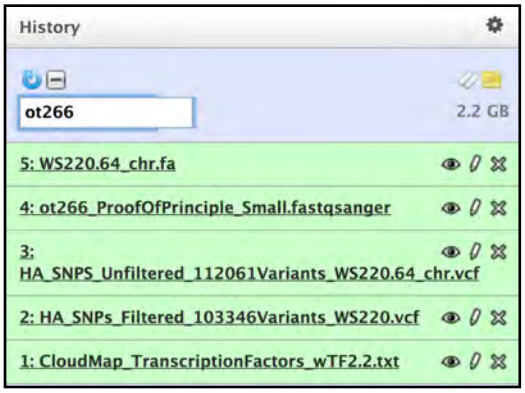
6) You will receive confirmation that the files have been imported into your history:

7) Click ***Analyze Data*** on the menu bar to navigate to your history:

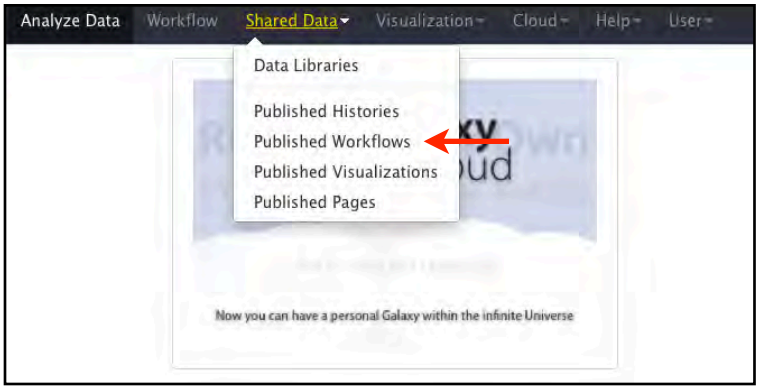
8) You will now see that the data files have been added to an unnamed history:



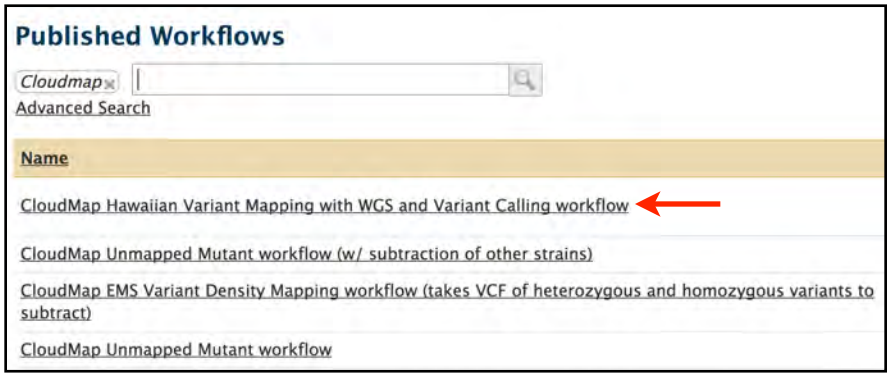
9) Name your history ***ot266*** after the sample that we will be analyzing:



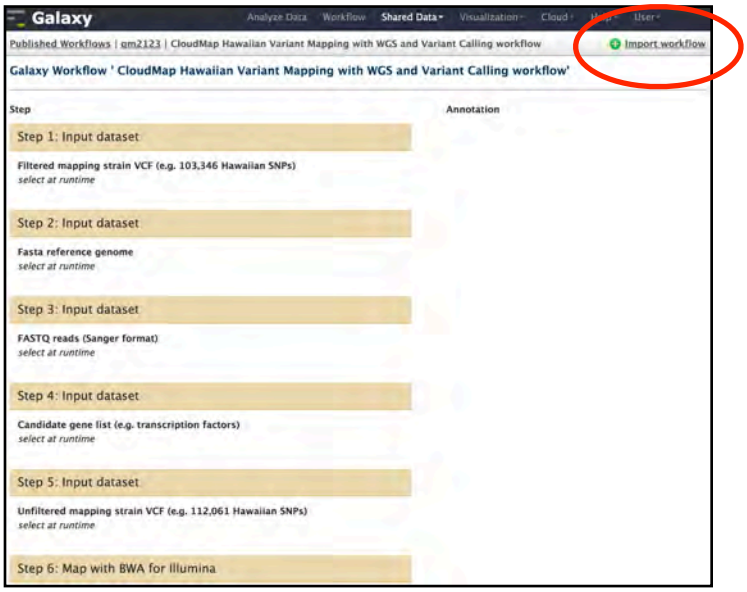
10) Again click on the ***Shared Data*** link at the top of the page and select ***Published Workflows*** :



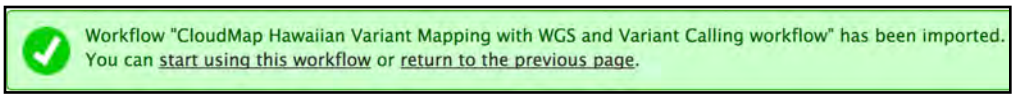
11) Use the search term “CloudMap” to view the automated workflows. Select the ***CloudMap Hawaiian Variant Mapping with WGS Data and Variant Calling workflow***.



12) You will now have the option to ***Import workflow***



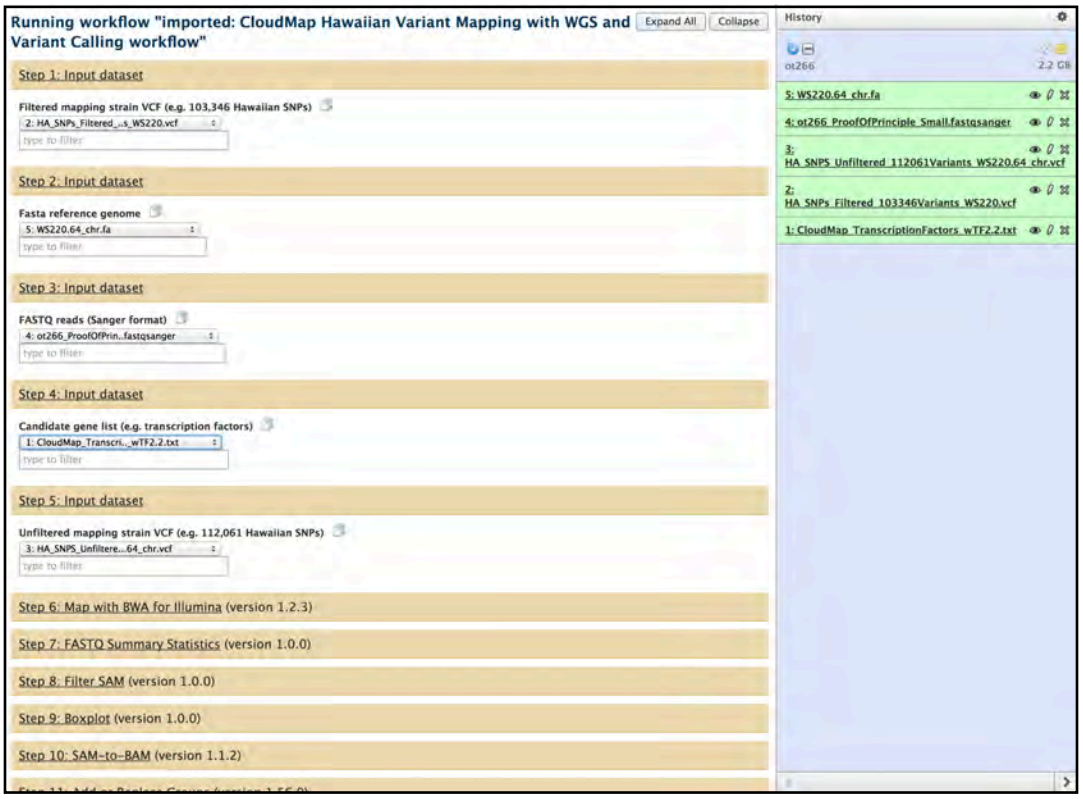
13) You will see the message below. Click ***Start using this workflow***.



14) You will see that the workflow has been imported. From now on, you can easily access this workflow under the ***Workflow*** tab.

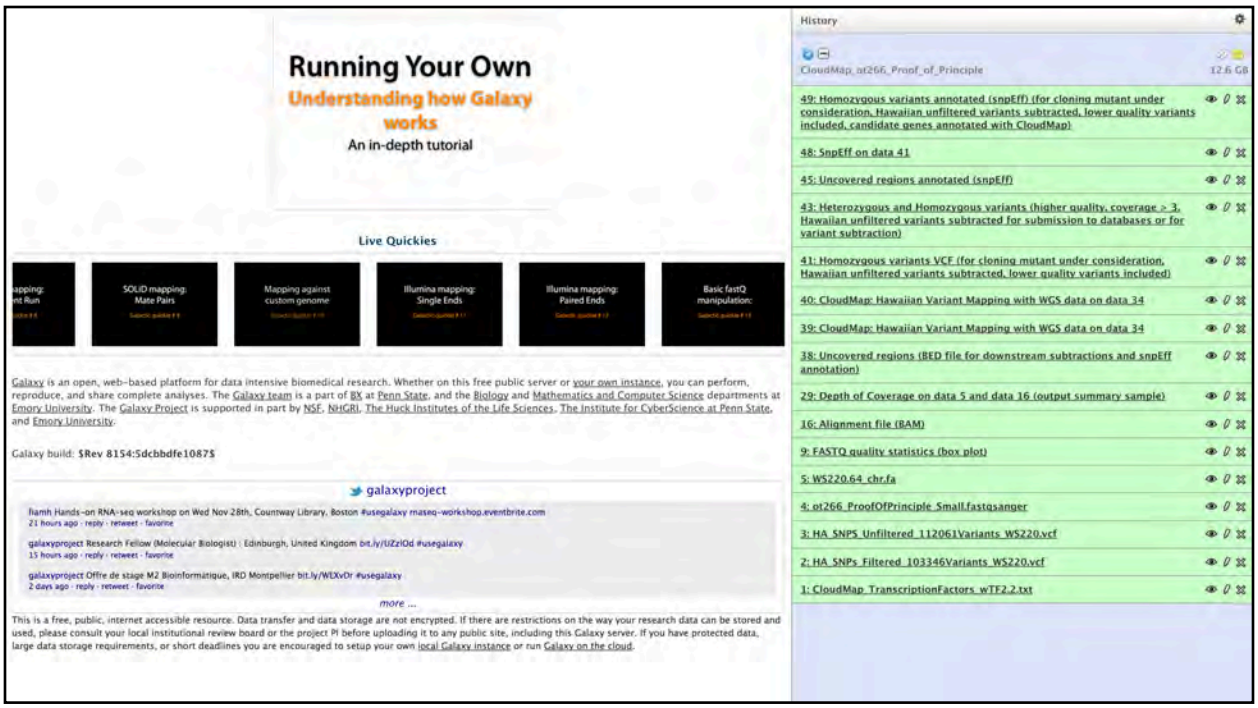
15) Click on the workflow and select ***Run***:

16) You will see all the steps in the workflow prior to running it. Make sure that each of the input fields corresponds to the appropriate file in your history.

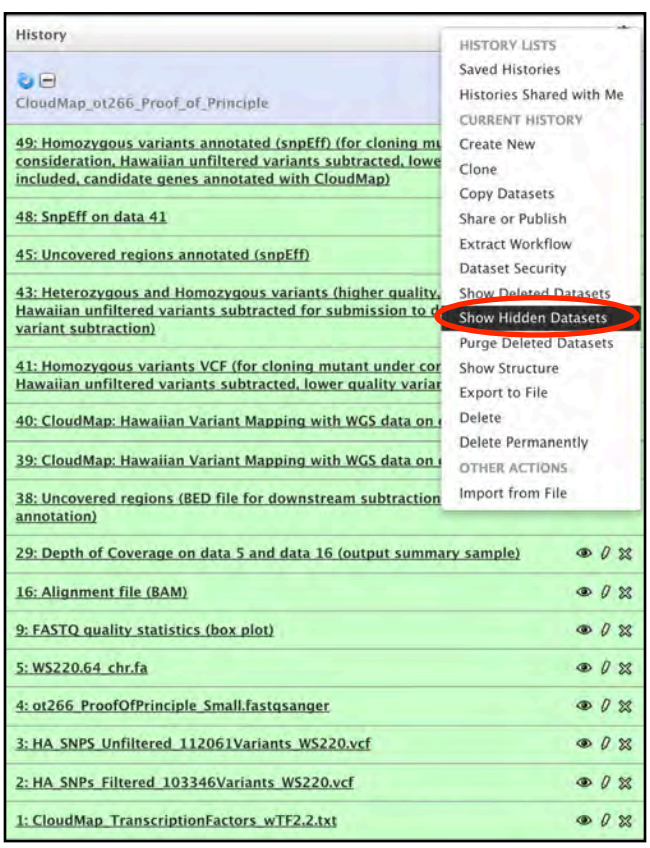


17) All of the automated functions have the appropriate default parameters configured, although experienced users may want to modify these prior to running (see the ***Analyzing Your Own Data Using CloudMap Workflows*** section of this user guide). Once you are ready to run the workflow, press ***Run Workflow*** at the bottom of the page and the workflow will start (this step takes a minute or two to begin, be patient and don't hit the ***Run Workflow*** button repeatedly). You will receive an email when the workflow is completed:

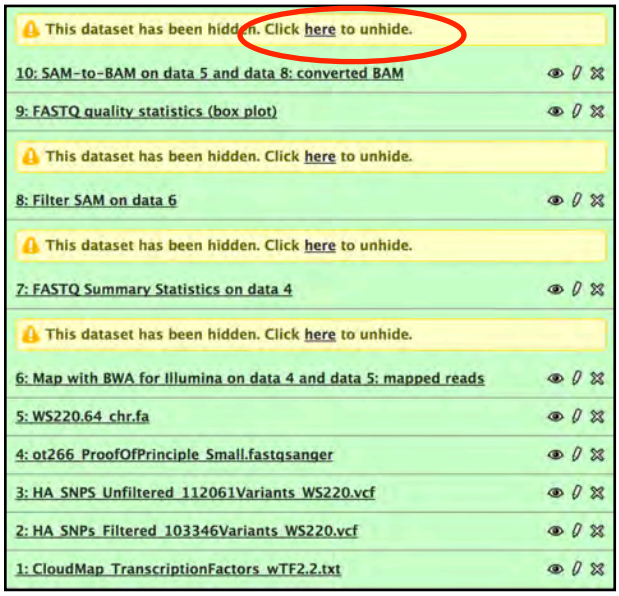
18) Once the workflow has finished running, you can view the resulting output:



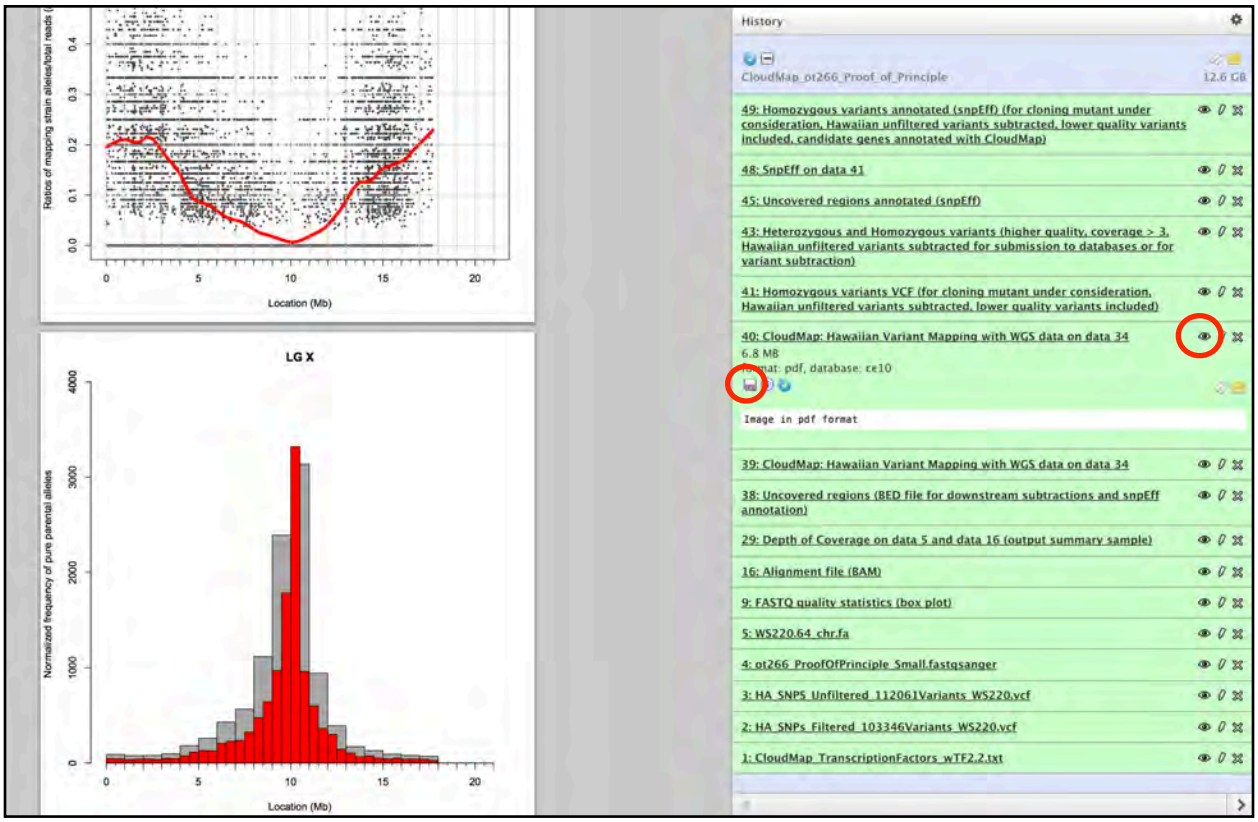
19) You will notice that while over 40 output files were generated during the course of the workflow (output files are sequentially numbered), only some output files remain visible while others are hidden. The visible files are most important for analysis of the mutant under consideration or downstream analysis. In order to view hidden files, click ***Show Hidden Datasets*** in the History menu:



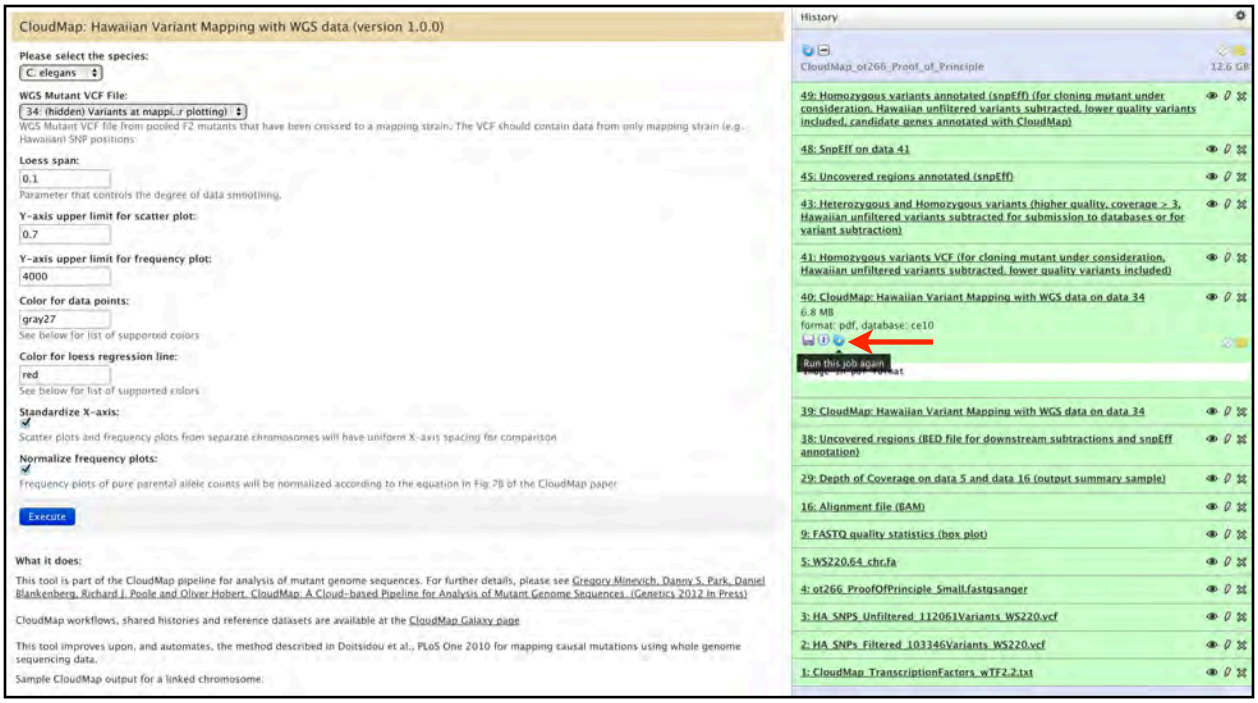
20) You may unhide any files that are hidden:



21) Click on a file to view more information on that file or to download the file:



If you want to rerun a tool with different parameters, click the ***run this job again*** arrow. To rerun a tool on a hidden dataset, make sure to unhide the hidden dataset first. If a tool fails (it will turn red) for no apparent reason when it has previously worked successfully, try running it again before submitting a bug report to Galaxy.



22) Several ***sample metric*** files are created as part of the workflow (more details on following pages):

1. A ***FASTQ quality statistics*** file summarizes the quality of all reads before they are aligned to the reference genome (*Galaxy's FASTQ manipulation tools*).

2. A ***Depth of Coverage*** file gives a summary of overall read depth in the BAM alignment file (*GATK*).

3. A ***graphical summary of all the variants*** in the sample (*snpEff)*. This file must be downloaded to be viewed properly. It will not appear correctly if viewed within Galaxy using the "peek" (eye) icon. (For more information on file format, see: http://snpeff.sourceforge.net/)

23) A ***primary set of files for analysis*** are created as part of the workflow:

1. A CloudMap-generated ***Hawaiian Variant Mapping plot*** that narrows down the region of genome containing the causal variant(s) and a ***tabular file containing the data used to make the plots***.

2. An ***annotated set of homozygous variants*** in the entire sample (*snpEff*) including annotation of candidate genes with CloudMap. (For more information on file format, see: http://snpeff.sourceforge.net/)

3. A ***BAM alignment file*** that can be viewed in your choice of alignment viewers (*SAMtools*). (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat.html)

4. A list of ***annotated uncovered regions*** (BED file) that may be putative deletions (*BEDtools* & *snpEff)*. (For more information on file format, see: http://snpeff.sourceforge.net/)

24) Additional files that can be used for ***downstream subtraction workflows*** are generated (for more details see the ***Subtract Variants*** and ***Uncovered Region Subtraction*** workflows):

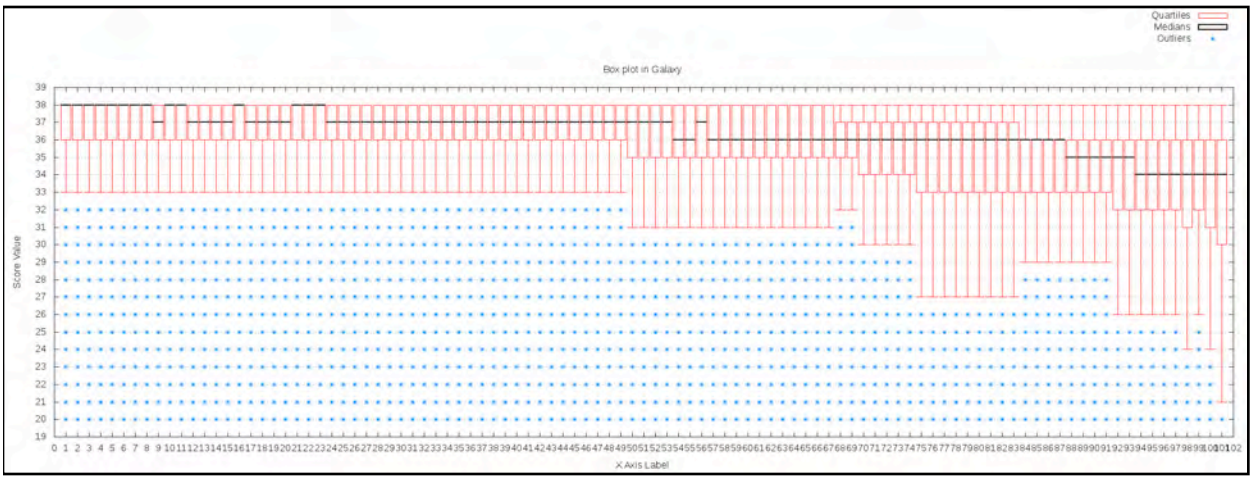
1. A ***set of homozygous variants*** (VCF file) in the entire sample that can be further filtered by subtracting variants present in other samples using the ***CloudMap Subtract Variants*** workflow (*GATK*). This VCF file is used as input into snpEff to generate the ***annotated list of homozygous variants*** mentioned in the section above. It has Hawaiian unfiltered variants subtracted and includes variants that pass a low quality filtering threshold. This file should be downloaded to be easily viewed in its entirety. The first several lines in any VCF file are header lines starting with "#" so users who wish to filter or sort these files in Excel are advised to remove the header lines. (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat.html)

2. A ***set of homozygous and heterozygous variants*** (VCF file) in the entire sample (run at higher quality stringency) that can be used as a set of variants to subtract from other samples (GATK). It has Hawaiian unfiltered variants subtracted and includes variants that pass a higher quality filtering threshold (read mapping quality ≥ 30 and coverage ≥ 3). In an effort to subtract as many variants as possible, users may subtract not only homozygous variants from other strains, but also heterozygous variants. Such a strategy assumes that phenotype-inducing homozygous mutant variants in the strain under analysis are unlikely to be heterozygous in strains that will be used for subtraction. It is especially important to apply this strategy when subtracting variant lists generated using the *Hawaiian Variant Mapping with WGS Data* approach (see section "**CloudMap *Hawaiian Variant Mapping with WGS Data*** tool"), since background variants will be present in a heterozygous state in these pooled samples as a consequence of the mapping cross. (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat.html)

3. A set of ***uncovered regions*** (BED file) used to generate the annotated uncovered regions mentioned in the section above. This list of uncovered regions can be used in two ways. It can be further filtered by subtracting uncovered regions present in other samples using the ***CloudMap Uncovered Region Subtraction*** workflow to find uncovered regions unique to the sample under analysis. The resultant file can then be annotated using snpEff. Alternatively, these uncovered regions can be used to subtract from the set of uncovered regions in other samples (using *BEDtools*). (for more details see the ***Subtract Variants*** and ***Uncovered Region Subtraction*** workflows) (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat.html)

Examples of ***sample metric*** files (mentioned in section 22 above):

22.1) ***FASTQ quality statistics*** file (*Galaxy's FASTQ manipulation tools*)



22.2) ***Depth of Coverage*** file (*GATK)*

	A	B	C	D	E	F	G
1	sample_id	total	mean	granular_third_quartile	granular_median	granular_first_quartile	%_bases_above_15
2	rgSM	734789704	7.33	11	7	4	9.7
3	Total	734789704	7.33	N/A	N/A	N/A	

22.3) ***Graphical summary of all the variants*** in the sample (html file from *snpEff)*. Note: this file is very comprehensive and only excerpts of it are shown here:

Contents

Number of effects by type and region

Type

Type (alphabetical order)	Count	Percent
CODON_INSERTION	1	0.001%
DOWNSTREAM	36,909	45.796%
FRAME_SHIFT	20	0.025%
INTERGENIC	22	0.027%
INTRON	4,139	5.136%
NON_SYNONYMOUS_CODING	724	0.898%
SPLICE_SITE_ACCEPTOR	3	0.004%
SPLICE_SITE_DONOR	1	0.001%
START_GAINED	13	0.016%
START_LOST	1	0.001%
STOP_GAINED	12	0.015%
SYNONYMOUS_CODING	711	0.882%
TRANSCRIPT	199	0.247%
UPSTREAM	37,618	46.675%
UTR_3_PRIME	137	0.17%
UTR_5_PRIME	85	0.105%

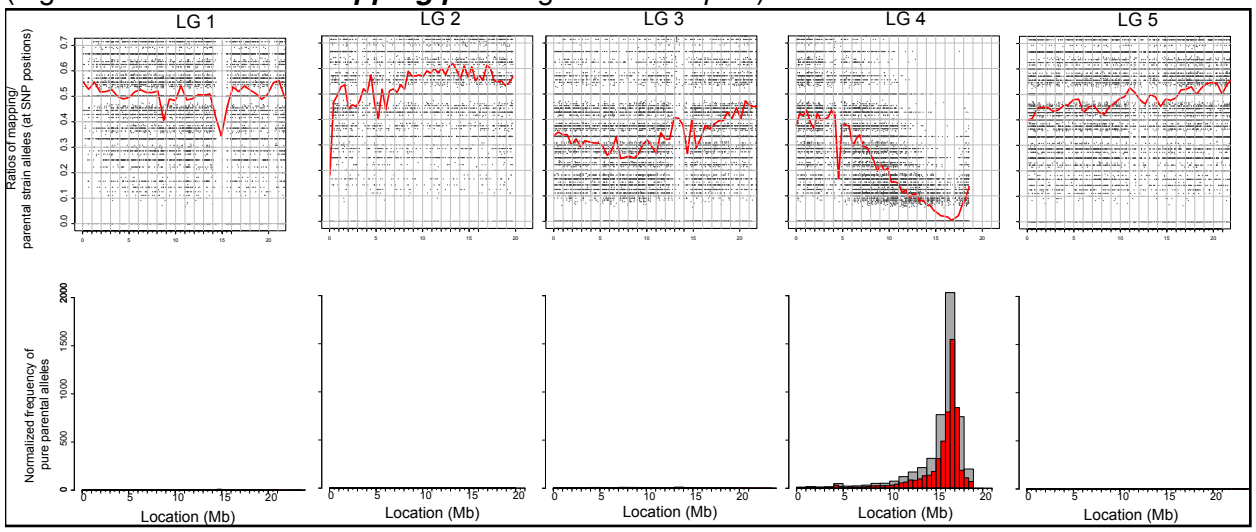
Region

Type (alphabetical order)	Count	Percent
DOWNSTREAM	36,909	45.796%
EXON	1,469	1.823%
INTERGENIC	22	0.027%
INTRON	4,139	5.136%
NONE	199	0.247%
SPLICE_SITE_ACCEPTOR	3	0.004%
SPLICE_SITE_DONOR	1	0.001%
UPSTREAM	37,618	46.675%
UTR_3_PRIME	137	0.17%
UTR_5_PRIME	98	0.122%

Examples of ***primary set of files for analysis*** (mentioned in step 23 above):

23.1) ***Hawaiian Variant Mapping plot*** and ***tabular file containing the data used to make the plots*** (*CloudMap*)

(e.g. ***Hawaiian Variant Mapping plot:*** Fig.10 *Arabidopsis*)



(e.g. ***Tabular file containing the data used to make the plots:*** *C. elegans*)

	A	B	C	D	E	F	G	H
1	#Chr	Pos	ID	Alt Count	Ref Count	Read Depth	Ratio	Mapping Unit
2	I	1222	haw1	4	3	7	0.571429	-21.9682
3	I	3659	haw3	6	7	13	0.461538	-21.9094
4	I	3731	haw4	4	11	15	0.266667	-21.9076
5	I	4101	haw5	9	12	21	0.428571	-21.8987
6	I	4776	haw6	1	8	9	0.111111	-21.8824
7	I	5026	haw7	4	10	14	0.285714	-21.8764
8	I	5868	haw8	0	5	5	0	-21.856

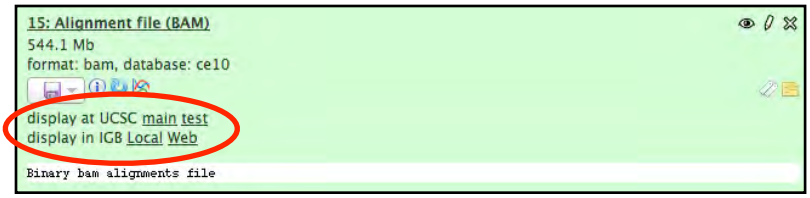
23.2) ***Annotated set of homozygous variants*** (Fig.4) (*snpEff*)

Fig. 4 : Sample screenshot of snpEff output

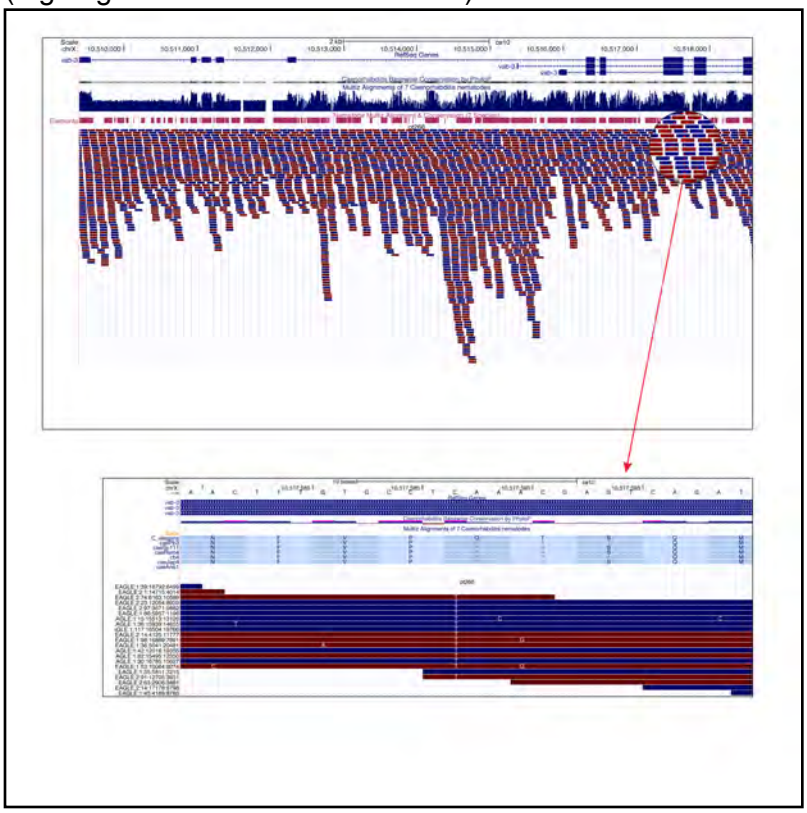
	# Chromo	Position	Reference	Change	Change_type	Quality	Coverage	Gene_ID	Gene_name	Bio_type	Transcript_ID	Exon_Rank	Effect	old_AA/new_AA	Old_codon/New_codon	Codon_Num(CDS)	CDS_size	
2	V	19885472 *		+G	INS	299.66	10	Y43F8B.17	Y43F8B.17	pseudogene	Y43F8B.17		TRANSCRIPT: Y43F8B.17				621	
3	X	2165878 *		+G	INS	2399.2	52	F48B9.3	F48B9.3	protein_codi	F48B9.3	5	FRAME_SHIFT: F48B9.3				585	
4	X	3412021 *		-T	DEL	196.55	25	C04F6.8	C04F6.8	ncRNA	C04F6.8		TRANSCRIPT: C04F6.8				134	
5	X	3903048 T		C	SNP	37.15	2	T22B2.11	T22B2.11	ncRNA	T22B2.11		TRANSCRIPT: T22B2.11				148	
6	X	6333449 C		T	SNP	157.66	5	S55D1.1	sgrm-2	protein_codi	S55D1.1	5	NON_SYNONYMOUS_CODING	G/R	Ggg/Agg	188	1911	
7	X	7037478 *		+G	INS	210.28	7	B0403.12	B0403.12	ncRNA	B0403.12		TRANSCRIPT: B0403.12				200	
8	X	7037478 *		+G	INS	210.28	7	B0403.13	B0403.13	ncRNA	B0403.13		TRANSCRIPT: B0403.13				203	
9	X	7310138 *		+C	INS	726.28	26	K03A1.1	K03A1.1	pseudogene	K03A1.1		TRANSCRIPT: K03A1.1				410	
10	X	7719013 *		+C	INS	635.6	22	K09F5.11	K09F5.11	ncRNA	K09F5.11		TRANSCRIPT: K09F5.11				137	
11	X	7719013 *		+C	INS	635.6	22	K09F5.10	K09F5.10	ncRNA	K09F5.10		TRANSCRIPT: K09F5.10				126	
12	X	7823447 *		+T	INS	300.36	16	R03G5.8	R03G5.8	ncRNA	R03G5.8		TRANSCRIPT: R03G5.8				161	
13	X	7866252 *		-A	DEL	1247.88	50	C54D2.16	C54D2.16	ncRNA	C54D2.16		TRANSCRIPT: C54D2.16				349	
14	X	8026796 *		+T	INS	317.94	10	C34D10.2	C34D10.2	protein_codi	C34D10.2.1		UTR_3_PRIME: 1423 bases from CDS					ZF - CCCH - Z domains
15	X	8292734 C		T	SNP	1085.02	41	F13B9.1	F13B9.1	protein_codi	F13B9.1b	14	NON_SYNONYMOUS_CODING	S/F	tCt/tTt	1426	4845	
16	X	8292734 C		T	SNP	1085.02	41	F13B9.1	F13B9.1	protein_codi	F13B9.1a	15	NON_SYNONYMOUS_CODING	S/F	tCt/tTt	1448	4899	
17	X	8292734 C		T	SNP	1085.02	41	F13B9.1	F13B9.1	protein_codi	F13B9.1c	14	NON_SYNONYMOUS_CODING	S/F	tCt/tTt	1426	4830	
18	X	8408774 *		+C	INS	476.87	12	F08F1.18	F08F1.18	ncRNA	F08F1.18		TRANSCRIPT: F08F1.18				283	
19	X	8639239 *		+CG	INS	775.11	16	F12D9.18	F12D9.18	ncRNA	F12D9.18		TRANSCRIPT: F12D9.18				88	
20	X	8639239 *		+CG	INS	775.11	16	F12D9.15	F12D9.15	tRNA	F12D9.15		TRANSCRIPT: F12D9.15				71	
21	X	8941351 *		-GATC	DEL	530.28	15	D1073.1	trk-1	protein_codi	D1073.1b	15	FRAME_SHIFT: D1073.1b				2523	
22	X	8941351 *		-GATC	DEL	530.28	15	D1073.1	trk-1	protein_codi	D1073.1a	12	FRAME_SHIFT: D1073.1a				2112	
23	X	9343610 *		+A	INS	654.81	30	T20B5.3	oga-1	protein_codi	T20B5.3a		UTR_3_PRIME: 75 bases from CDS					
24	X	10482433 C		T	SNP	1776.49	42	C33D3.1	alt-2	protein_codi	C33D3.1	7	NON_SYNONYMOUS_CODING	S/F	tCt/tTt	811	1802	ZF - GATA
25	X	10517587 C		T	SNP	376.64	16	F14F3.1	vab-3	protein_codi	F14F3.1b	4	STOP_GAINED	Q/*	Caa/Taa	152	810	HD - PRD, Paired Domain - FULL
26	X	10517587 C		T	SNP	376.64	16	F14F3.1	vab-3	protein_codi	F14F3.1a	9	STOP_GAINED	Q/*	Caa/Taa	338	1368	HD - PRD, Paired Domain - FULL
27	X	10517587 C		T	SNP	376.64	16	F14F3.1	vab-3	protein_codi	F14F3.1c	4	STOP_GAINED	Q/*	Caa/Taa	179	891	HD - PRD, Paired Domain - FULL
28	X	11660051 C		T	SNP	572.86	22	T04F8.1	sfen-1.5	protein_codi	T04F8.1	5	NON_SYNONYMOUS_CODING	G/R	Gga/Aga	214	975	
29	X	11695533 C		T	SNP	427.81	19	C44C10.4	C44C10.4	protein_codi	C44C10.4	7	NON_SYNONYMOUS_CODING	I/F	Atc/Ttc	535	1634	
30	X	12492661 *		+G	INS	681.86	18	F45E6.7	F45E6.7	ncRNA	F45E6.7		TRANSCRIPT: F45E6.7				145	
31	X	14060338 T		C	SNP	85.86	3	C33G3.13	C33G3.13	ncRNA	C33G3.13		TRANSCRIPT: C33G3.13				71	
32	X	14305870 C		T	SNP	1288.01	46	C11H1.2	C11H1.2	protein_codi	C11H1.2	7	SYNONYMOUS_CODING	K/K	aaG/aaA	252	1383	
33	X	16608728 *		-AG	DEL	809.66	24	F59C12.8	F59C12.8	ncRNA	F59C12.8		TRANSCRIPT: F59C12.8				225	
34	X	17259200 T		C	SNP	45.01	14	Y40C7B.3	Y40C7B.3	protein_codi	Y40C7B.3	1	SYNONYMOUS_CODING	V/V	gtA/gtG	104	1251	

23.3) ***BAM alignment*** file (*SAMtools*) (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat.html)

Click on the "***display in"*** link in your history or download the BAM file to view it in your alignment viewer of choice:



(e.g. Fig.9 UCSC Genome Browser)



Note: Information displayed in alignment viewers often will not exactly match that in variant files (VCFs) or lists of annotated variants (snpEff). This is because read mapping qualities and base qualities are incorporated into which variants are ultimately called. Most alignment viewers have filter settings that can be used to only display reads with mapping quality scores above a certain value. Applying these filters should result in alignments that more closely approximate variant lists.

23.4) A list of ***annotated uncovered regions*** (BED file) (*BEDtools* & *snpEff)* (For more information on file format, see: http://snpeff.sourceforge.net/)

	A	B	C	D	E	F	G	H	I	J
1	# Chromo	Position	Reference	Homozygous	Coverage	Gene_name	Bio_type	Trancript_ID	Exon_ID	old_AA/new_AA
2	I	2646	2664	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.4	UPSTREAM: 8859 bases
3	I	2646	2664	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.6	UPSTREAM: 8972 bases
4	I	2646	2664	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.3	UPSTREAM: 7767 bases
5	I	2646	2664	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.2	UPSTREAM: 8849 bases
6	I	2646	2664	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.1	UPSTREAM: 8853 bases
7	I	2646	2664	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.5	UPSTREAM: 8853 bases
8	I	2646	2664	Interval	0	Y74C9A.3	Y74C9A.3	protein_coding	Y74C9A.3.1	DOWNSTREAM: 1473 bases
9	I	2646	2664	Interval	0	Y74C9A.3	Y74C9A.3	protein_coding	Y74C9A.3.2	DOWNSTREAM: 1575 bases
10	I	2646	2664	Interval	0	Y74C9A.6	Y74C9A.6	snoRNA	Y74C9A.6	DOWNSTREAM: 1101 bases
11	I	3468	3482	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.4	UPSTREAM: 8037 bases
12	I	3468	3482	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.6	UPSTREAM: 8150 bases
13	I	3468	3482	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.3	UPSTREAM: 6945 bases
14	I	3468	3482	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.2	UPSTREAM: 8027 bases
15	I	3468	3482	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.1	UPSTREAM: 8031 bases
16	I	3468	3482	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.5	UPSTREAM: 8031 bases
17	I	3468	3482	Interval	0	Y74C9A.3	Y74C9A.3	protein_coding	Y74C9A.3.1	DOWNSTREAM: 651 bases
18	I	3468	3482	Interval	0	Y74C9A.3	Y74C9A.3	protein_coding	Y74C9A.3.2	DOWNSTREAM: 753 bases
19	I	3468	3482	Interval	0	Y74C9A.6	Y74C9A.6	snoRNA	Y74C9A.6	DOWNSTREAM: 279 bases
20	I	3926	4014	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.4	UPSTREAM: 7579 bases
21	I	3926	4014	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.6	UPSTREAM: 7692 bases
22	I	3926	4014	Interval	0	Y74C9A.6	Y74C9A.6	snoRNA	Y74C9A.6	UPSTREAM: 17 bases
23	I	3926	4014	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.3	UPSTREAM: 6487 bases

Additional files that can be used for ***downstream subtraction workflows*** (mentioned in step 24 above):

24.1) ***Set of homozygous variants*** (VCF file generated by *GATK*). Header lines starting with "#" have been removed in Excel. (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat)

	A	B	C	D	E	F	G	H	I	J	K
1	#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	rgSM	
2	chrI	42899	.	G	A	75.03	PASS	AC=2;AF=1.00;AN=2;DP=3;	GT:AD:DP:GQ:PL	1/1:0,3:3:9.03:107,9,0	
3	chrI	62642	.	T	C	48.77	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.02:80,6,0	
4	chrI	341299	.	TG	T	181.31	PASS	AC=2;AF=1.00;AN=2;DP=6;	GT:AD:DP:GQ:PL	1/1:0,6:6:18.06:223,18,0	
5	chrI	346149	.	T	A	85.77	PASS	AC=2;AF=1.00;AN=2;DP=3;	GT:AD:DP:GQ:PL	1/1:0,3:3:9.03:118,9,0	
6	chrI	361325	.	C	A	232.91	PASS	AC=2;AF=1.00;AN=2;DP=7;	GT:AD:DP:GQ:PL	1/1:0,7:7:21.07:266,21,0	
7	chrI	369870	.	C	T	48.08	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.02:79,6,0	
8	chrI	369871	.	C	T	48.77	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.02:80,6,0	
9	chrI	663697	.	G	C	167.29	PASS	AC=2;AF=1.00;AN=2;DP=5;	GT:AD:DP:GQ:PL	1/1:0,5:5:15.05:200,15,0	
10	chrI	670146	.	G	A	36.43	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.01:68,6,0	
11	chrI	670173	.	T	C	36.43	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.01:68,6,0	
12	chrI	671425	.	T	A	48.77	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.02:80,6,0	
13	chrI	687402	.	T	A	67.01	PASS	AC=2;AF=1.00;AN=2;DP=3;	GT:AD:DP:GQ:PL	1/1:0,3:3:9.01:99,9,0	

24.2) ***Set of homozygous and heterozygous variants*** (VCF file generated by *GATK*). Header lines starting with "#" have been removed in Excel. (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat)

	A	B	C	D	E	F	G	H	I	J
1	#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	rgSM
2	chrI	962	.	G	T	367.18	.	AC=1;AF=0.50;AN=2;BaseQRankSum=0.403;DP=23	GT:AD:DP:GQ:PL	0/1:10,13:23:99:397,0,325
3	chrI	991	.	GA	G	100.41	.	AC=1;AF=0.50;AN=2;BaseQRankSum=2.130;DP=14	GT:AD:DP:GQ:PL	0/1:8,6:14:99:139,0,246
4	chrI	1216	.	A	T	68.96	.	AC=1;AF=0.50;AN=2;BaseQRankSum=1.300;DP=7;	GT:AD:DP:GQ:PL	0/1:4,3:7:98.95:99,0,138
5	chrI	1222	.	A	C	109.76	.	AC=1;AF=0.50;AN=2;BaseQRankSum=1.754;DP=7;	GT:AD:DP:GQ:PL	0/1:3,4:7:57.20:140,0,57
6	chrI	1290	.	T	A	126.47	.	AC=1;AF=0.50;AN=2;BaseQRankSum=0.933;DP=14	GT:AD:DP:GQ:PL	0/1:9,5:14:99:156,0,306
7	chrI	1412	.	T	C	235.12	.	AC=1;AF=0.50;AN=2;BaseQRankSum=-1.203;DP=1	GT:AD:DP:GQ:PL	0/1:8,9:17:99:265,0,266
8	chrI	1414	.	G	A	205.1	.	AC=1;AF=0.50;AN=2;BaseQRankSum=-0.209;DP=1	GT:AD:DP:GQ:PL	0/1:7,8:15:99:235,0,233
9	chrI	1421	.	G	A	196.85	.	AC=1;AF=0.50;AN=2;BaseQRankSum=-1.096;DP=1	GT:AD:DP:GQ:PL	0/1:7,8:15:99:227,0,228

24.3) ***Set of uncovered regions (***BED file***)*** (*BEDtools*). (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat)

	A	B	C	D
1	chrI	2645	2664	0
2	chrI	3467	3482	0
3	chrI	3925	4014	0
4	chrI	8673	8703	0
5	chrI	8835	8995	0
6	chrI	9774	9787	0
7	chrI	11219	11317	0
8	chrI	11450	11469	0
9	chrI	15107	15117	0
10	chrI	15635	15767	0

Note: We strongly suggest that users employ the ***Subtract Variants*** and ***Uncovered Region Subtraction*** workflows if additional strains are available for this purpose. The general concept is shown in **Fig.5** of the CloudMap paper.

CloudMap | Cloud-based Pipeline for Analysis of Mutant Genome Sequences

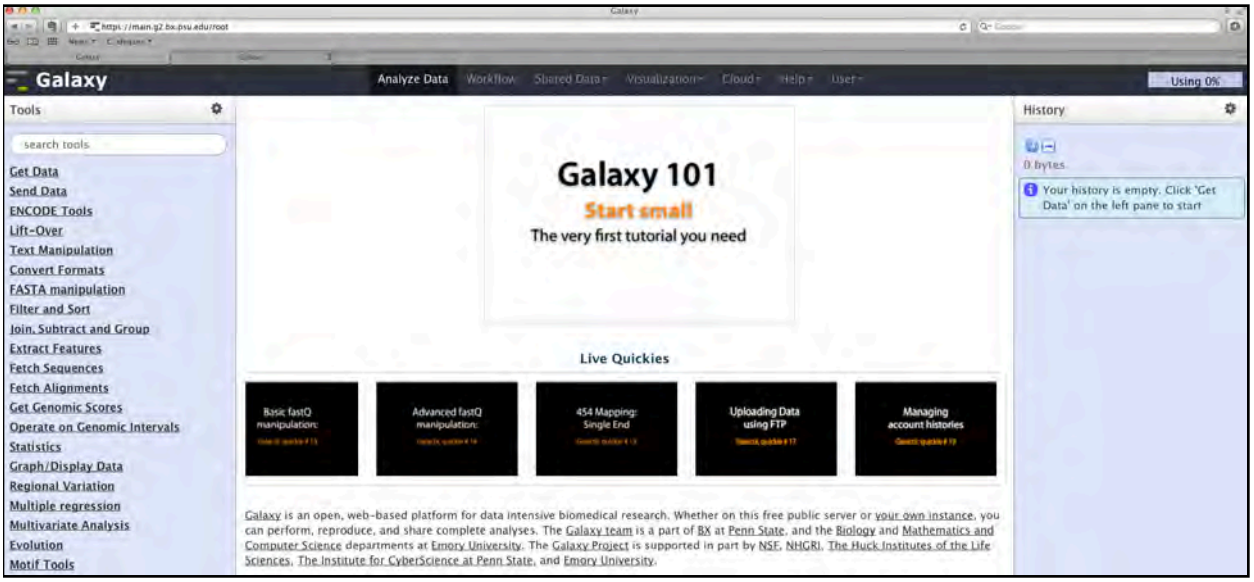
CloudMap UnMapped Mutant Workflow

This workflow performs the same analysis as the ***Hawaiian Variant Mapping with WGS data and Variant Calling workflow*** without the mapping-specific tools and input reference files. The workflow should be used for data generated from a single mutant, not from pooled mutants resulting from a cross to a mapping strain. This workflow uses single-end FASTQ data but it can be adapted to use paired-end data (see the ***Analyzing Your Own Data*** section of this user guide). A video version of this user guide is available at: http://usegalaxy.org/cloudmap.

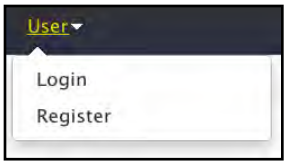
These workflows provide default function parameters, ensuring that users follow best practices, and allow for automated execution of sequential operations. We provide these workflows as helpful guides, but experienced users may execute functions in any meaningful order they please and may also create and share their own workflows to take advantage of the automation feature. More CloudMap documentation is available at http://usegalaxy.org/cloudmap.

The *ot266* FASTQ file used in this example represents Hawaiian variant mapped data but for the purposes of this user guide, we perform an unmapped analysis. Users wishing to run their own unmapped data should also view the ***Analyzing Your Own Data*** section of this user guide before proceeding.

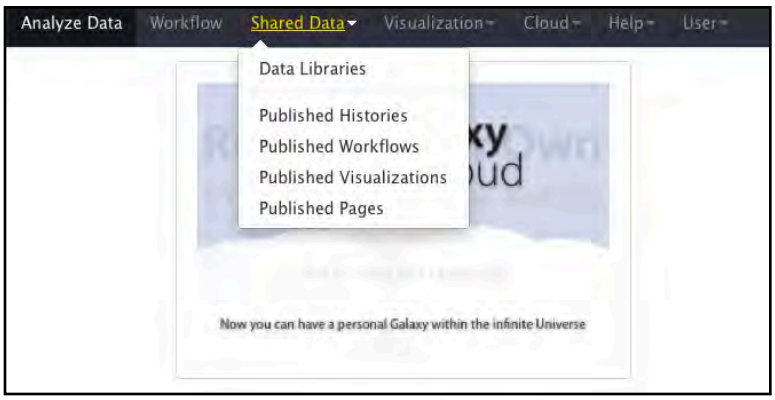
1) Navigate to http://usegalaxy.org (URL will resolve to something like https://main.g2.bx.psu.edu)



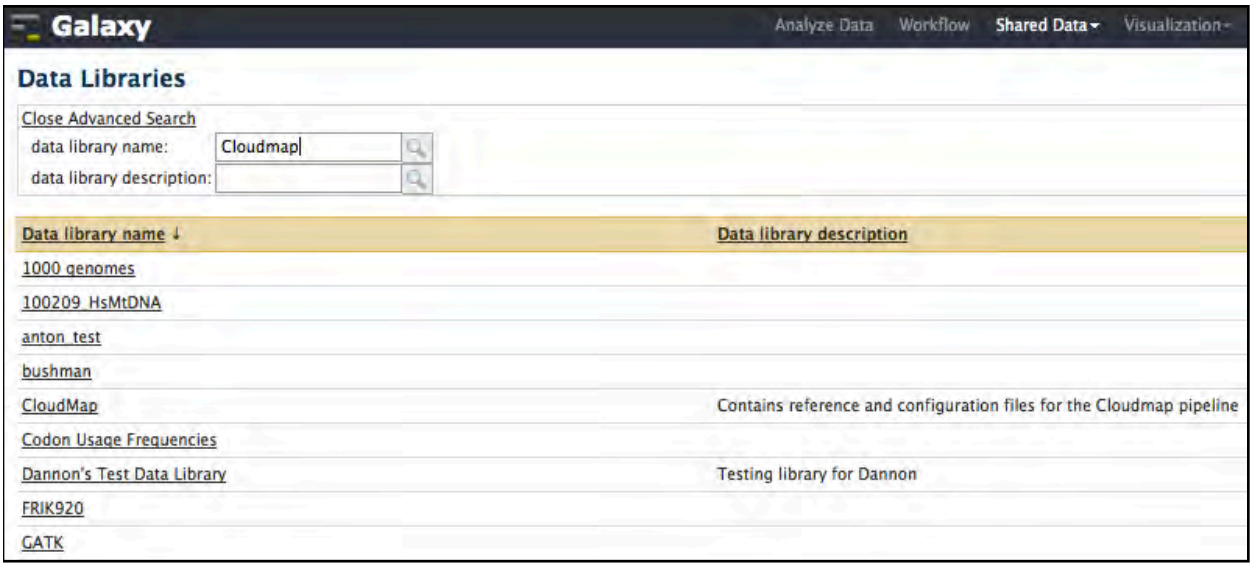
2) Register for an account or login if you already have an account:



3) Once you are logged in using your email address, click on the ***Shared Data*** link at the top of the page:



4) Click on ***Data Libraries*** and search for the CloudMap data library:



5) Click on the ***CloudMap*** library and select the 5 data files below for the *ot266* example. Then click "Go" to import these files into your history.

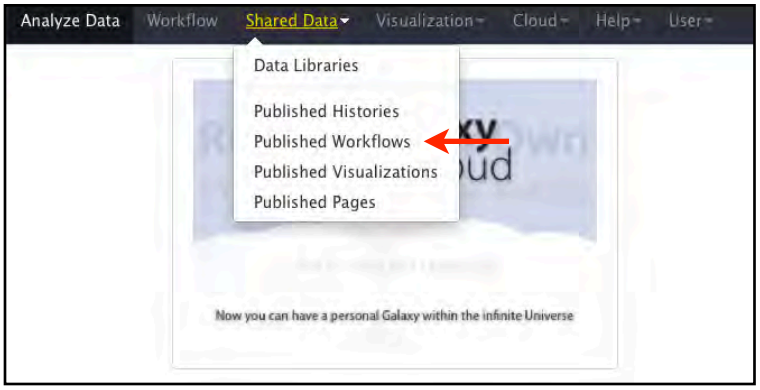
6) You will receive confirmation that the files have been imported into your history:

7) Click ***Analyze Data*** on the menu bar to navigate to your history:

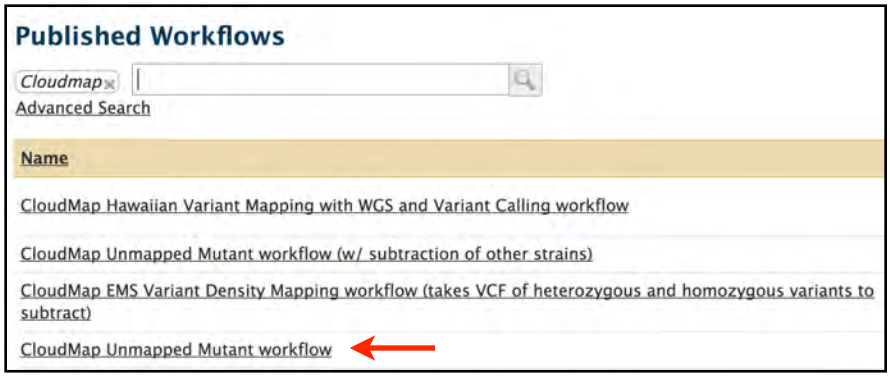
8) You will now see that the data files have been added to an unnamed history:

9) Name your history ***ot266*** after the sample that we will be analyzing:

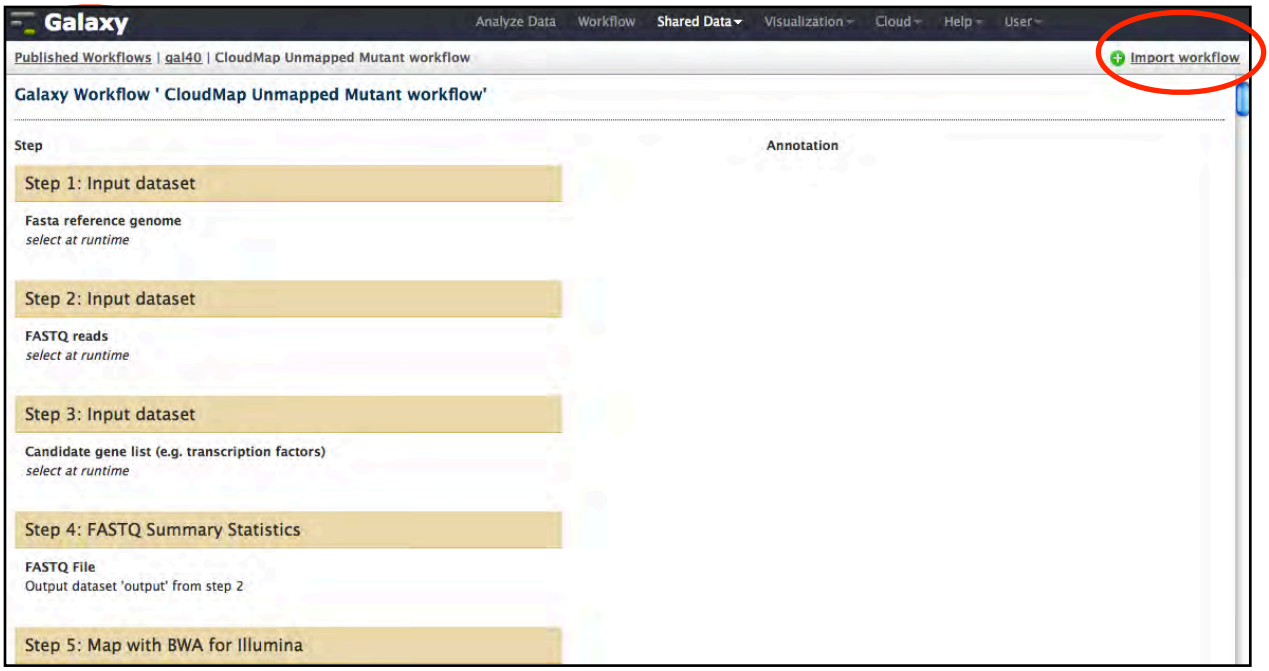
10) Again click on the ***Shared Data*** link at the top of the page and select ***Published Workflows*** :



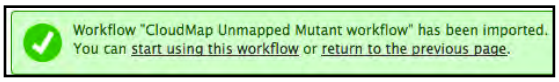
11) Use the search term “CloudMap” to view the automated workflows. Select the ***CloudMap Unmapped Mutant workflow.***



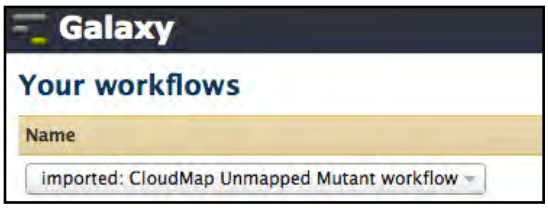
12) You will now have the option to ***Import workflow***



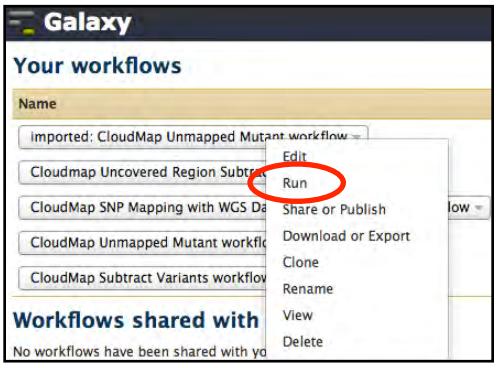
13) You will see the message below. Click ***Start using this workflow***.



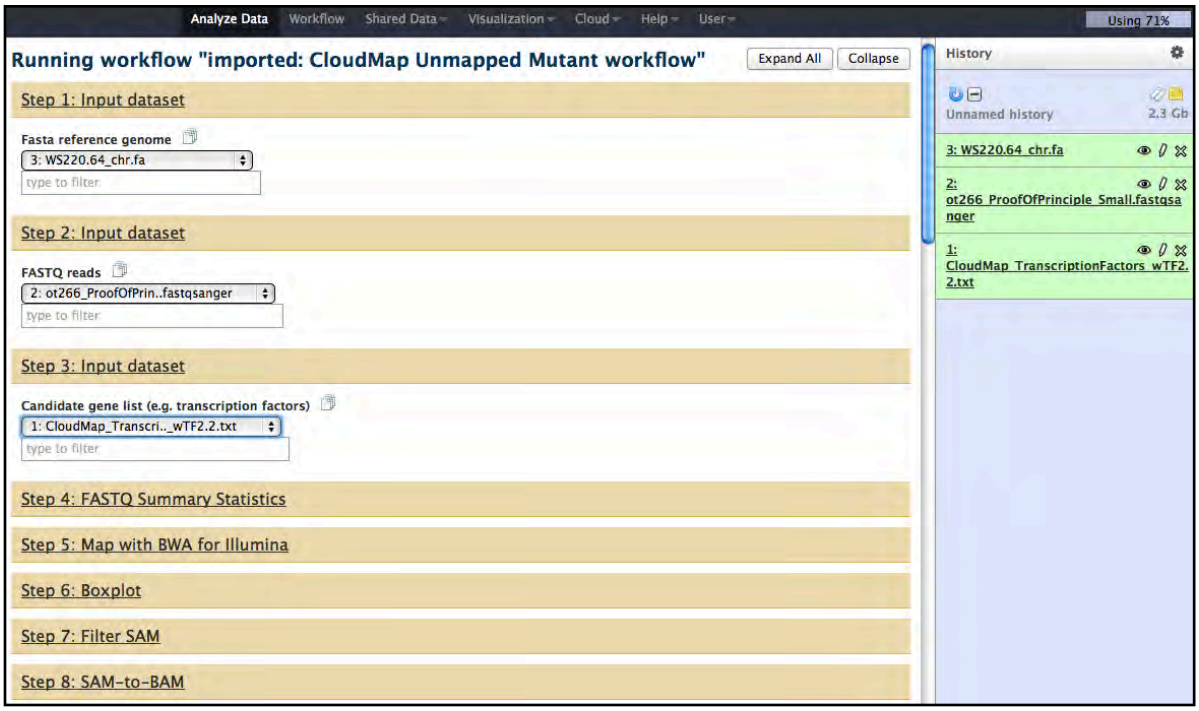
14) You will see that the workflow has been imported. From now on, you can easily access this workflow under the ***Workflow*** tab.



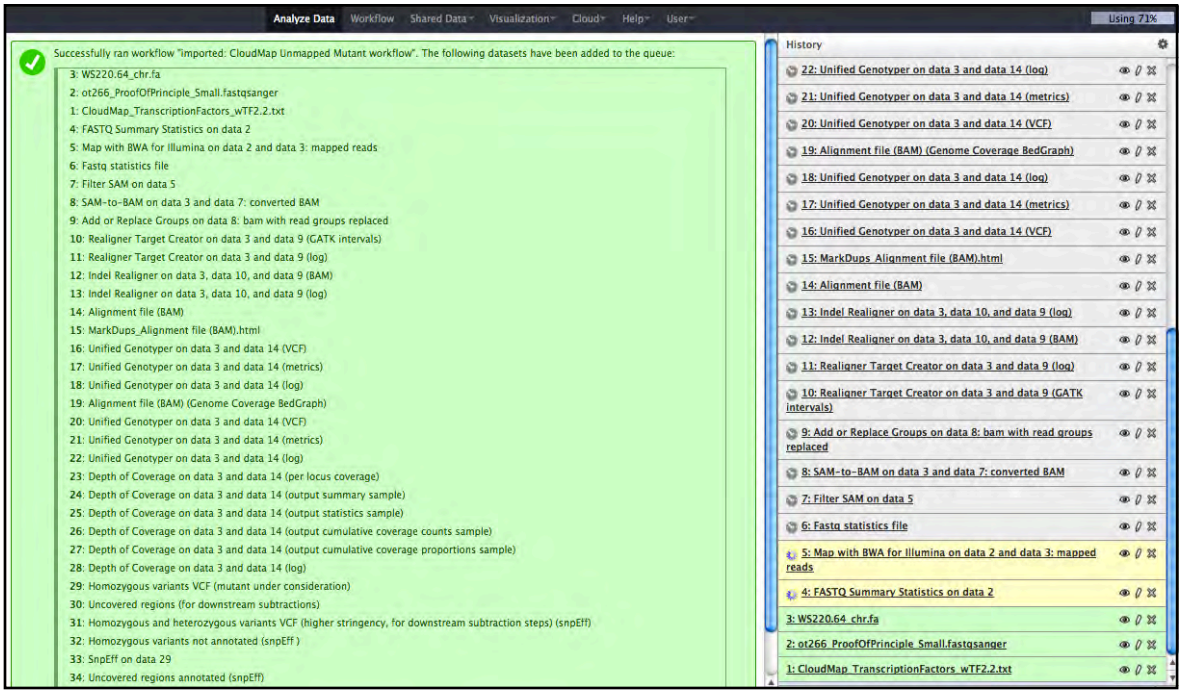
15) Click on the workflow and select ***Run***:



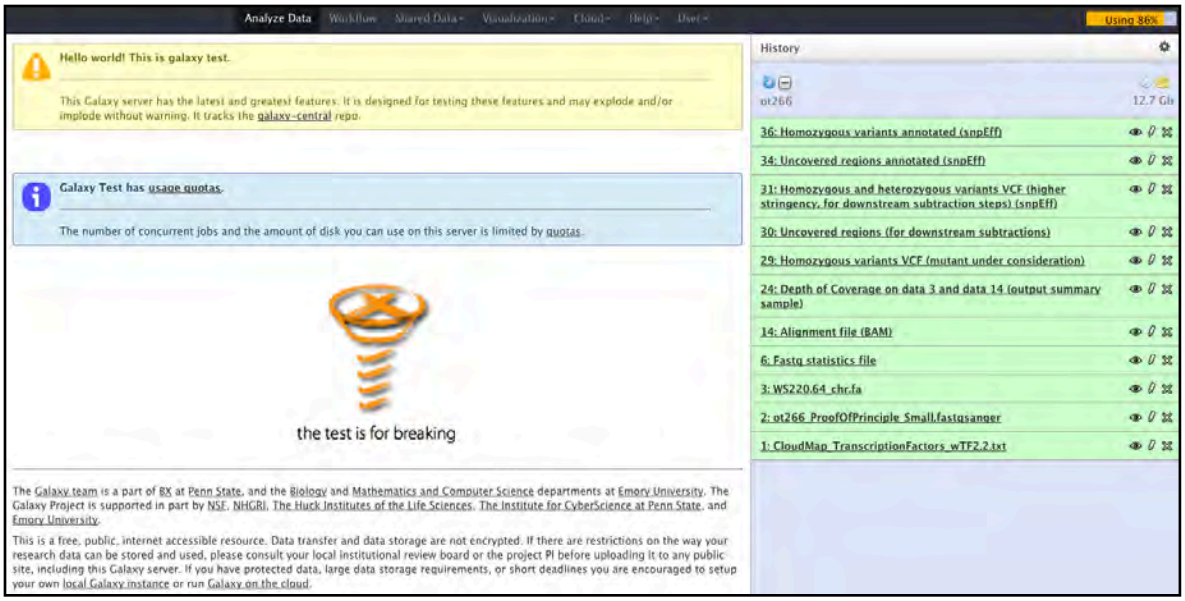
16) You will see all the steps in the workflow prior to running it. Make sure that each of the input fields corresponds to the appropriate file in your history.



17) All of the automated functions have the appropriate default parameters configured, although experienced users may want to modify these prior to running (see the ***Analyzing Your Own Data Using CloudMap Workflows*** section of this user guide). Once you are ready to run the workflow, press ***Run Workflow*** at the bottom of the page and the workflow will start (this step takes a minute or two to begin, be patient and don't hit the ***Run Workflow*** button repeatedly). You will receive an email when the workflow is completed:

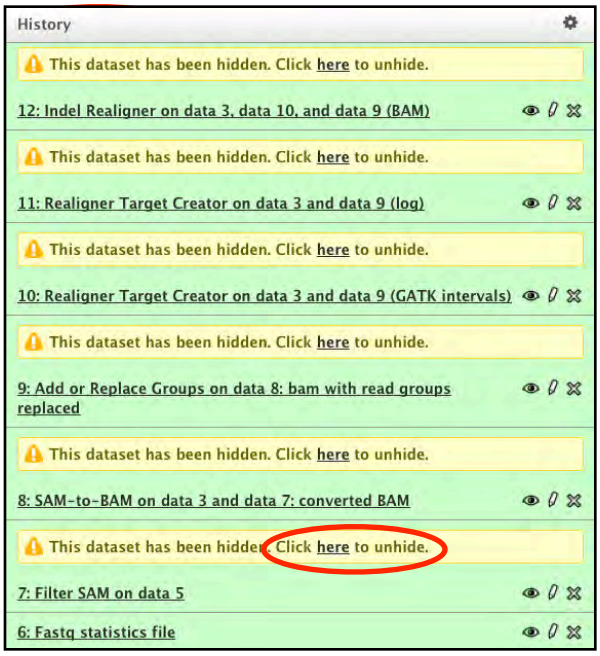


18) Once the workflow has finished running, you can view the resulting output:

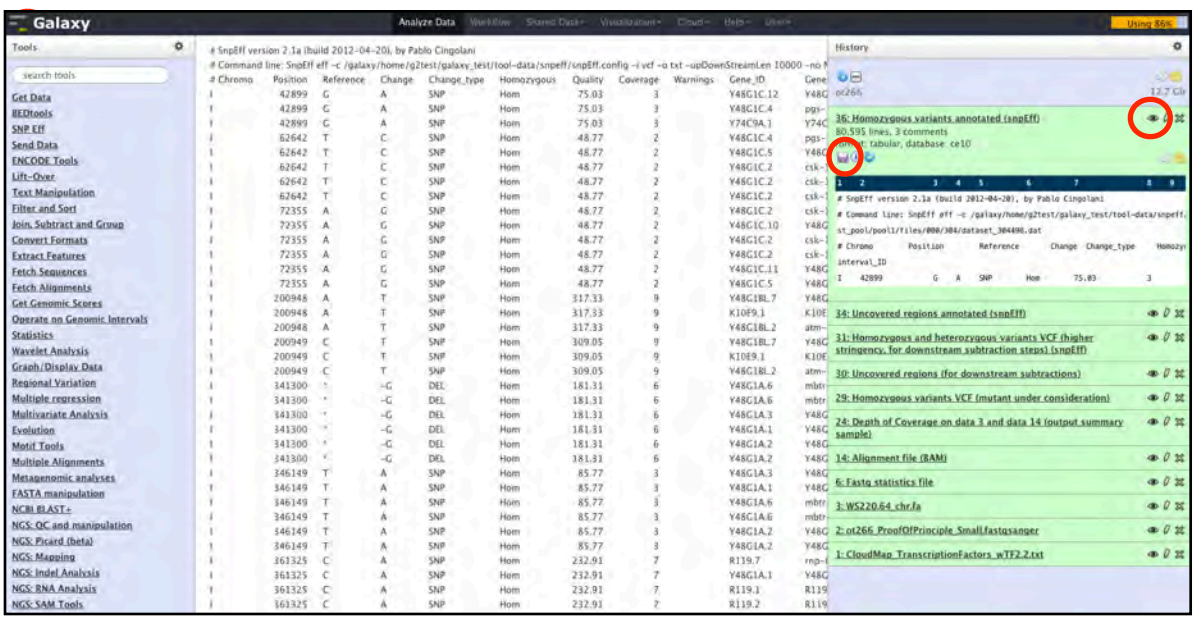


19) You will notice that while over 30 output files were generated during the course of the workflow (output files are sequentially numbered), only some output files remain visible while others are hidden. The visible files are most important for analysis of the mutant under consideration or downstream analysis. In order to view hidden files, click ***Show Hidden Datasets*** in the History menu:

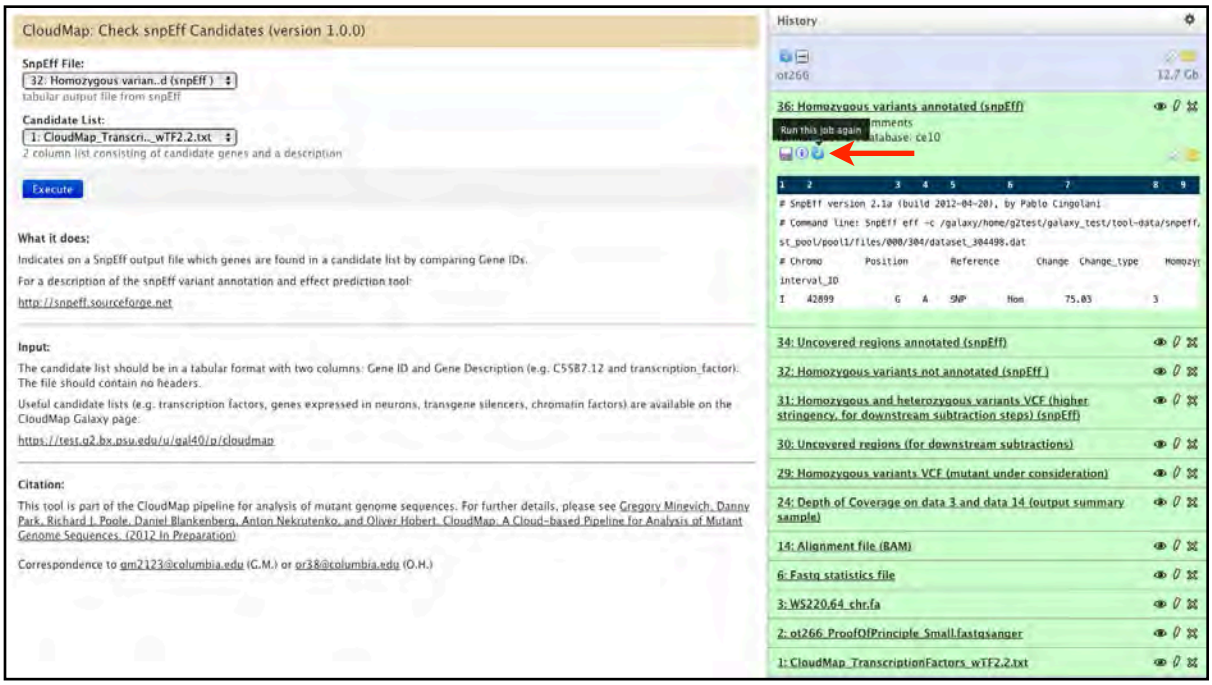
20) You may unhide any files that are hidden:



21) Click on a file to view more information on that file or to download the file:



22) If you want to rerun a tool with different parameters, click the ***run this job again*** arrow. To rerun a tool on a hidden dataset, make sure to unhide the hidden dataset first. If a tool fails (it will turn red) for no apparent reason when it has previously worked successfully, try running it again before submitting a bug report to Galaxy.



23) Several ***sample metric*** files are created as part of the workflow (more details on following pages):

1. A ***FASTQ quality statistics*** file summarizes the quality of all reads before they are aligned to the reference genome (*Galaxy's FASTQ manipulation tools*).

2. A ***Depth of Coverage*** file gives a summary of overall read depth in the BAM alignment file (*GATK*).

3. A ***graphical summary of all the variants*** in the sample (*snpEff)*. This file must be downloaded to be viewed properly. It will not appear correctly if viewed within Galaxy using the "peek" (eye) icon. (For more information on file format, see: http://snpeff.sourceforge.net/)

24) A ***primary set of files for analysis*** are created as part of the workflow:

1. An ***annotated set of homozygous variants*** in the entire sample (*snpEff*). (For more information on file format, see: http://snpeff.sourceforge.net/)

2. A ***BAM alignment file*** that can be viewed in your choice of alignment viewers (*SAMtools*). (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat)

3. A list of ***annotated uncovered regions*** (BED file) that may be putative deletions (*BEDtools* & *snpEff)*. (For more information on file format, see: http://snpeff.sourceforge.net/)

25) Additional files that can be used for ***downstream subtraction workflows*** are generated (for more details see the ***Subtract Variants*** and ***Uncovered Region Subtraction*** workflows):

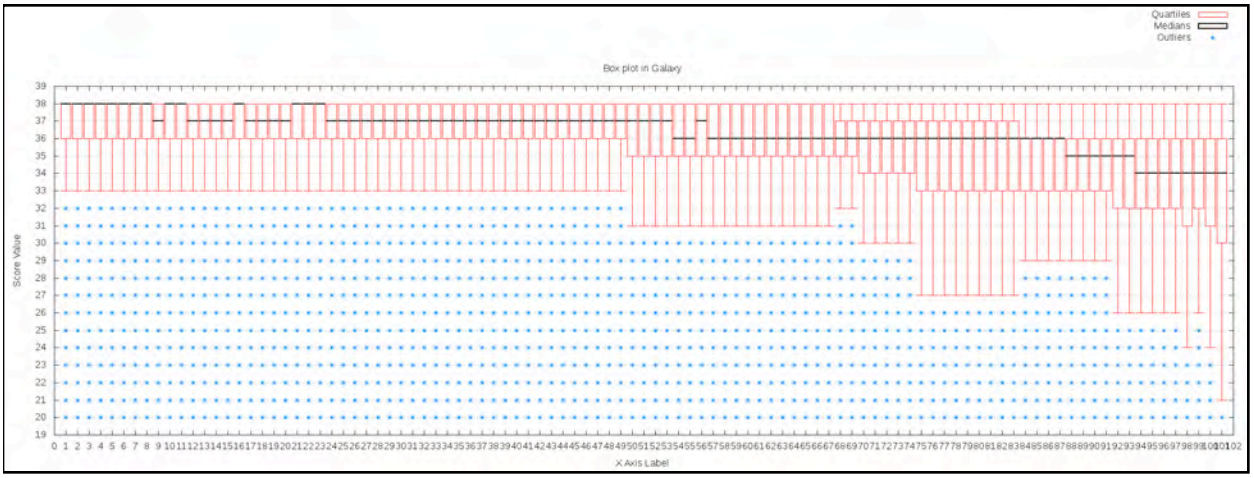
1. A ***set of homozygous variants*** (VCF file) in the entire sample that can be further filtered by subtracting variants present in other samples using the ***CloudMap Subtract Variants*** workflow (*GATK*). This VCF file is used as input into snpEff to generate the ***annotated list of homozygous variants*** mentioned in the section above. It has Hawaiian unfiltered variants subtracted and includes variants that pass a low quality filtering threshold. This file should be downloaded to be easily viewed in its entirety. The first several lines in any VCF file are header lines starting with "#" so users who wish to filter or sort these files in Excel are advised to remove the header lines. (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat.html)

2. A ***set of homozygous and heterozygous variants*** (VCF file) in the entire sample (run at higher quality stringency) that can be used as a set of variants to subtract from other samples (GATK). It has Hawaiian unfiltered variants subtracted and includes variants that pass a higher quality filtering threshold (read mapping quality ≥ 30 and coverage ≥ 3). In an effort to subtract as many variants as possible, users may subtract not only homozygous variants from other strains, but also heterozygous variants. Such a strategy assumes that phenotype-inducing homozygous mutant variants in the strain under analysis are unlikely to be heterozygous in strains that will be used for subtraction. It is especially important to apply this strategy when subtracting variant lists generated using the *Hawaiian Variant Mapping with WGS Data* approach (see section "**CloudMap *Hawaiian Variant Mapping with WGS Data*** tool"), since background variants will be present in a heterozygous state in these pooled samples as a consequence of the mapping cross. (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat.html)

3. A set of ***uncovered regions*** (BED file) used to generate the annotated uncovered regions mentioned in the section above. This list of uncovered regions can be used in two ways. It can be further filtered by subtracting uncovered regions present in other samples using the ***CloudMap Uncovered Region Subtraction*** workflow to find uncovered regions unique to the sample under analysis. The resultant file can then be annotated using snpEff. Alternatively, these uncovered regions can be used to subtract from the set of uncovered regions in other samples (using *BEDtools*). (for more details see the ***Subtract Variants*** and ***Uncovered Region Subtraction*** workflows) (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat.html)

Examples of ***sample metric*** files (mentioned in section 22 above):

23.1) ***FASTQ quality statistics*** file (*Galaxy's FASTQ manipulation tools*)



23.2) ***Depth of Coverage*** file (*GATK)*

	A	B	C	D	E	F	G
1	sample_id	total	mean	granular_third_quartile	granular_median	granular_first_quartile	%_bases_above_15
2	rgSM	734789704	7.33	11	7	4	9.7
3	Total	734789704	7.33	N/A	N/A	N/A	

23.3) ***Graphical summary of all the variants*** in the sample (html file from *snpEff)*. Note: this file is very comprehensive and only excerpts of it are shown here:

Contents

Number of effects by type and region

Type

Type (alphabetical order)	Count	Percent
CODON_INSERTION	1	0.001%
DOWNSTREAM	36,909	45.796%
FRAME_SHIFT	20	0.025%
INTERGENIC	22	0.027%
INTRON	4,139	5.136%
NON_SYNONYMOUS_CODING	724	0.898%
SPLICE_SITE_ACCEPTOR	3	0.004%
SPLICE_SITE_DONOR	1	0.001%
START_GAINED	13	0.016%
START_LOST	1	0.001%
STOP_GAINED	12	0.015%
SYNONYMOUS_CODING	711	0.882%
TRANSCRIPT	199	0.247%
UPSTREAM	37,618	46.675%
UTR_3_PRIME	137	0.17%
UTR_5_PRIME	85	0.105%

Region

Type (alphabetical order)	Count	Percent
DOWNSTREAM	36,909	45.796%
EXON	1,469	1.823%
INTERGENIC	22	0.027%
INTRON	4,139	5.136%
NONE	199	0.247%
SPLICE_SITE_ACCEPTOR	3	0.004%
SPLICE_SITE_DONOR	1	0.001%
UPSTREAM	37,618	46.675%
UTR_3_PRIME	137	0.17%
UTR_5_PRIME	98	0.122%

Examples of ***primary set of files for analysis*** (mentioned in step 23 above):

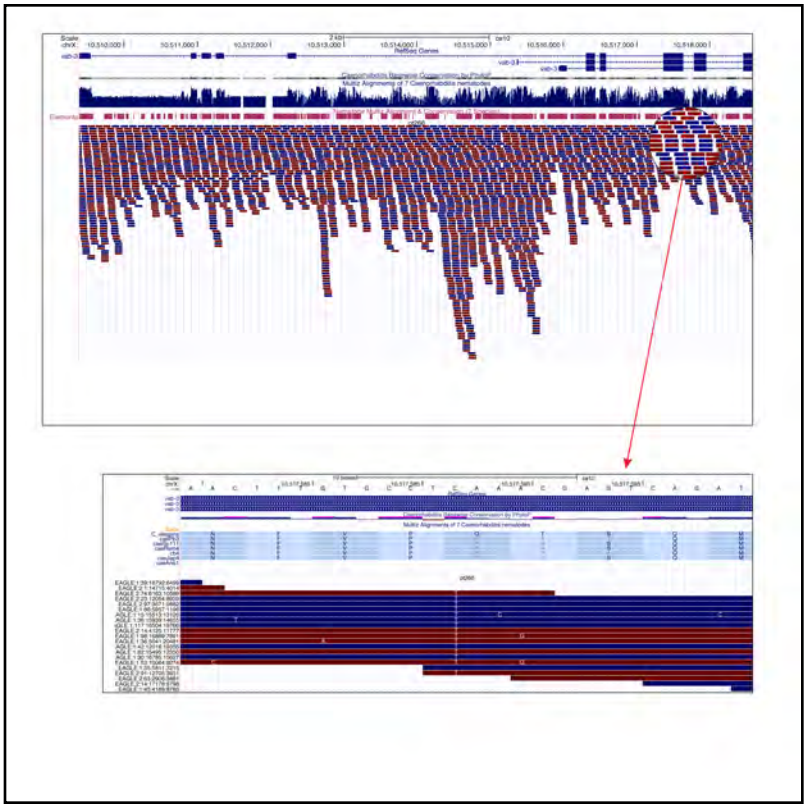
24.1) ***Annotated set of homozygous variants*** (Fig.4) (*snpEff*)

Fig. 4 : Sample screenshot of snpEff output

# Chromo	Position	Reference	Change	Change_type	Quality	Coverage	Gene_ID	Gene_name	Bio_type	Transcript_ID	Exon_Rank	Effect	old_AA/new_AA	Old_codon/New_codon	Codon_Num(CDS)	CDS_size	
V	19485472	*	+G	INS	299.66	10	Y43F8B.17	Y43F8B.17	pseudogene	Y43F8B.17		TRANSCRIPT: Y43F8B.17				621	
X	2165878	*	+G	INS	2399.2	52	F48B9.3	F48B9.3	protein_codi	F48B9.3	5	FRAME_SHIFT: F48B9.3				585	
X	3412021	*	-T	DEL	196.55	25	C04F6.8	C04F6.8	ncRNA	C04F6.8		TRANSCRIPT: C04F6.8				124	
X	3903048	T	C	SNP	37.15	2	T22B2.11	T22B2.11	ncRNA	T22B2.11		TRANSCRIPT: T22B2.11				148	
X	6383449	C	T	SNP	157.66	5	SSSD1.1	igcm-2	protein_codi	SSSD1.1	5	NON_SYNONYMOUS_CODING	G/R	Ggg/Agg	188	1911	
X	7037478	*	+G	INS	210.28	7	B0403.12	B0403.12	ncRNA	B0403.12		TRANSCRIPT: B0403.12				200	
X	7037478	*	+G	INS	210.28	7	B0403.13	B0403.13	ncRNA	B0403.13		TRANSCRIPT: B0403.13				203	
X	7310138	*	+C	INS	726.28	26	K03A1.1	K03A1.1	pseudogene	K03A1.1		TRANSCRIPT: K03A1.1				410	
X	7719013	*	+C	INS	635.6	22	K09F5.11	K09F5.11	ncRNA	K09F5.11		TRANSCRIPT: K09F5.11				137	
X	7719013	*	+C	INS	635.6	22	K09F5.10	K09F5.10	ncRNA	K09F5.10		TRANSCRIPT: K09F5.10				126	
X	7823447	*	+T	INS	300.36	16	R03G5.8	R03G5.8	ncRNA	R03G5.8		TRANSCRIPT: R03G5.8				141	
X	7866252	*	-A	DEL	1247.88	50	C54D2.16	C54D2.16	ncRNA	C54D2.16		TRANSCRIPT: C54D2.16				349	
X	8026796	*	+T	INS	317.94	10	C34D10.2	C34D10.2	protein_codi	C34D10.2.1		UTR_3_PRIME: 1423 bases from CDS					ZF - CCCH - 2 domains
X	8292734	C	T	SNP	1085.02	41	F13B9.1	F13B9.1	protein_codi	F13B9.1b	14	NON_SYNONYMOUS_CODING	S/F	tCt/tTt	1426	4845	
X	8292734	C	T	SNP	1085.02	41	F13B9.1	F13B9.1	protein_codi	F13B9.1a	15	NON_SYNONYMOUS_CODING	S/F	tCt/tTt	1448	4899	
X	8292734	C	T	SNP	1085.02	41	F13B9.1	F13B9.1	protein_codi	F13B9.1c	14	NON_SYNONYMOUS_CODING	S/F	tCt/tTt	1426	4830	
X	8408774	*	+C	INS	476.87	12	F08F1.18	F08F1.18	ncRNA	F08F1.18		TRANSCRIPT: F08F1.18				283	
X	8639239	*	+CG	INS	775.11	16	F12D9.18	F12D9.18	ncRNA	F12D9.18		TRANSCRIPT: F12D9.18				88	
X	8639239	*	+CG	INS	775.11	16	F12D9.15	F12D9.15	tRNA	F12D9.15		TRANSCRIPT: F12D9.15				71	
X	8941351	*	-GATC	DEL	530.28	15	D1073.1	trk-1	protein_codi	D1073.1b	15	FRAME_SHIFT: D1073.1b				2523	
X	8941351	*	-GATC	DEL	530.28	15	D1073.1	trk-1	protein_codi	D1073.1a	12	FRAME_SHIFT: D1073.1a				2112	
X	9343610	*	+A	INS	654.81	30	T20B5.3	sgk-1	protein_codi	T20B5.3a		UTR_3_PRIME: 75 bases from CDS					
X	10482433	C	T	SNP	1776.49	42	C31D3.1	elt-2	protein_codi	C31D3.1	7	NON_SYNONYMOUS_CODING	S/F	tCt/tTt	311	1802	ZF - GATA
X	10517587	C	T	SNP	376.64	16	F14F3.1	vab-3	protein_codi	F14F3.1b	4	STOP_GAINED	Q/*	Caa/Taa	152	810	HD - PRD, Paired Domain - FULL
X	10517587	C	T	SNP	376.64	16	F14F3.1	vab-3	protein_codi	F14F3.1a	9	STOP_GAINED	Q/*	Caa/Taa	338	1368	HD - PRD, Paired Domain - FULL
X	10517587	C	T	SNP	376.64	16	F14F3.1	vab-3	protein_codi	F14F3.1c	4	STOP_GAINED	Q/*	Caa/Taa	179	891	HD - PRD, Paired Domain - FULL
X	11660051	C	T	SNP	572.86	22	T04F8.1	tfm-1.5	protein_codi	T04F8.1	5	NON_SYNONYMOUS_CODING	G/R	Gga/Aga	214	975	
X	11695533	C	T	SNP	427.81	19	C44C10.4	C44C10.4	protein_codi	C44C10.4	7	NON_SYNONYMOUS_CODING	I/F	Ctc/Ttc	535	1614	
X	12492661	*	+G	INS	631.86	18	F45E6.7	F45E6.7	ncRNA	F45E6.7		TRANSCRIPT: F45E6.7				145	
X	14068938	T	C	SNP	85.86	3	C33G3.13	C33G3.13	ncRNA	C33G3.13		TRANSCRIPT: C33G3.13				71	
X	14305870	C	T	SNP	1288.01	46	C11H1.2	C11H1.2	protein_codi	C11H1.2	7	SYNONYMOUS_CODING	K/K	aaG/aaA	252	1383	
X	16608728	*	-AC	DEL	809.66	24	F59C12.8	F59C12.8	ncRNA	F59C12.8		TRANSCRIPT: F59C12.8				225	
X	17259200	T	C	SNP	45.01	14	Y40C7B.3	Y40C7B.3	protein_codi	Y40C7B.3	1	SYNONYMOUS_CODING	V/V	gtA/gtG	104	1251	

24.2) ***BAM alignment*** file (*SAMtools*) (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat.html). Click on the "***display in"*** link in your history or download the BAM file to view it in your alignment viewer of choice:

(e.g. Fig.9 UCSC Genome Browser)



Note: Information displayed in alignment viewers often will not exactly match that in variant files (VCFs) or lists of annotated variants (snpEff). This is because read mapping qualities and base qualities are incorporated into which variants are ultimately called. Most alignment viewers have filter settings that can be used to only display reads with mapping quality scores above a certain value. Applying these filters should result in alignments that more closely approximate variant lists.

24.3) A list of ***annotated uncovered regions*** (BED file) (*BEDtools* & *snpEff)* (For more information on file format, see: http://snpeff.sourceforge.net/)

	A	B	C	D	E	F	G	H	I	J
1	# Chromo	Position	Reference	Homozygous	Coverage	Gene_name	Bio_type	Trancript_ID	Exon_ID	old_AA/new_AA
2	I	2646	2664	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.4	UPSTREAM: 8859 bases
3	I	2646	2664	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.6	UPSTREAM: 8972 bases
4	I	2646	2664	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.3	UPSTREAM: 7767 bases
5	I	2646	2664	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.2	UPSTREAM: 8849 bases
6	I	2646	2664	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.1	UPSTREAM: 8853 bases
7	I	2646	2664	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.5	UPSTREAM: 8853 bases
8	I	2646	2664	Interval	0	Y74C9A.3	Y74C9A.3	protein_coding	Y74C9A.3.1	DOWNSTREAM: 1473 bases
9	I	2646	2664	Interval	0	Y74C9A.3	Y74C9A.3	protein_coding	Y74C9A.3.2	DOWNSTREAM: 1575 bases
10	I	2646	2664	Interval	0	Y74C9A.6	Y74C9A.6	snoRNA	Y74C9A.6	DOWNSTREAM: 1101 bases
11	I	3468	3482	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.4	UPSTREAM: 8037 bases
12	I	3468	3482	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.6	UPSTREAM: 8150 bases
13	I	3468	3482	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.3	UPSTREAM: 6945 bases
14	I	3468	3482	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.2	UPSTREAM: 8027 bases
15	I	3468	3482	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.1	UPSTREAM: 8031 bases
16	I	3468	3482	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.5	UPSTREAM: 8031 bases
17	I	3468	3482	Interval	0	Y74C9A.3	Y74C9A.3	protein_coding	Y74C9A.3.1	DOWNSTREAM: 651 bases
18	I	3468	3482	Interval	0	Y74C9A.3	Y74C9A.3	protein_coding	Y74C9A.3.2	DOWNSTREAM: 753 bases
19	I	3468	3482	Interval	0	Y74C9A.6	Y74C9A.6	snoRNA	Y74C9A.6	DOWNSTREAM: 279 bases
20	I	3926	4014	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.4	UPSTREAM: 7579 bases
21	I	3926	4014	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.6	UPSTREAM: 7692 bases
22	I	3926	4014	Interval	0	Y74C9A.6	Y74C9A.6	snoRNA	Y74C9A.6	UPSTREAM: 17 bases
23	I	3926	4014	Interval	0	Y74C9A.2	nlp-40	protein_coding	Y74C9A.2.3	UPSTREAM: 6487 bases

Additional files that can be used for ***downstream subtraction workflows*** (mentioned in step 25 above):

25.1) ***Set of homozygous variants*** (VCF file generated by *GATK*). Header lines starting with "#" have been removed in Excel. (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat)

	A	B	C	D	E	F	G	H	I	J	K
1	#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	rgSM	
2	chrI	42899	.	G	A	75.03	PASS	AC=2;AF=1.00;AN=2;DP=3;	GT:AD:DP:GQ:PL	1/1:0,3:3:9.03:107,9,0	
3	chrI	62642	.	T	C	48.77	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.02:80,6,0	
4	chrI	341299	.	TG	T	181.31	PASS	AC=2;AF=1.00;AN=2;DP=6;	GT:AD:DP:GQ:PL	1/1:0,6:6:18.06:223,18,0	
5	chrI	346149	.	T	A	85.77	PASS	AC=2;AF=1.00;AN=2;DP=3;	GT:AD:DP:GQ:PL	1/1:0,3:3:9.03:118,9,0	
6	chrI	361325	.	C	A	232.91	PASS	AC=2;AF=1.00;AN=2;DP=7;	GT:AD:DP:GQ:PL	1/1:0,7:7:21.07:266,21,0	
7	chrI	369870	.	C	T	48.08	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.02:79,6,0	
8	chrI	369871	.	C	T	48.77	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.02:80,6,0	
9	chrI	663697	.	G	C	167.29	PASS	AC=2;AF=1.00;AN=2;DP=5;	GT:AD:DP:GQ:PL	1/1:0,5:5:15.05:200,15,0	
10	chrI	670146	.	G	A	36.43	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.01:68,6,0	
11	chrI	670173	.	T	C	36.43	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.01:68,6,0	
12	chrI	671425	.	T	A	48.77	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.02:80,6,0	
13	chrI	687402	.	T	A	67.01	PASS	AC=2;AF=1.00;AN=2;DP=3;	GT:AD:DP:GQ:PL	1/1:0,3:3:9.01:99,9,0	

25.2) ***Set of homozygous and heterozygous variants*** (VCF file generated by *GATK*). Header lines starting with "#" have been removed in Excel. (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat)

	A	B	C	D	E	F	G	H	I	J
1	#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	rgSM
2	chrI	962	.	G	T	367.18	.	AC=1;AF=0.50;AN=2;BaseQRankSum=0.403;DP=23	GT:AD:DP:GQ:PL	0/1:10,13:23:99:397,0,325
3	chrI	991	.	GA	G	100.41	.	AC=1;AF=0.50;AN=2;BaseQRankSum=2.130;DP=14	GT:AD:DP:GQ:PL	0/1:8,6:14:99:139,0,246
4	chrI	1216	.	A	T	68.96	.	AC=1;AF=0.50;AN=2;BaseQRankSum=1.300;DP=7;	GT:AD:DP:GQ:PL	0/1:4,3:7:98.95:99,0,138
5	chrI	1222	.	A	C	109.76	.	AC=1;AF=0.50;AN=2;BaseQRankSum=1.754;DP=7;	GT:AD:DP:GQ:PL	0/1:3,4:7:57.20:140,0,57
6	chrI	1290	.	T	A	126.47	.	AC=1;AF=0.50;AN=2;BaseQRankSum=0.933;DP=14	GT:AD:DP:GQ:PL	0/1:9,5:14:99:156,0,306
7	chrI	1412	.	T	C	235.12	.	AC=1;AF=0.50;AN=2;BaseQRankSum=-1.203;DP=1	GT:AD:DP:GQ:PL	0/1:8,9:17:99:265,0,266
8	chrI	1414	.	G	A	205.1	.	AC=1;AF=0.50;AN=2;BaseQRankSum=-0.209;DP=1	GT:AD:DP:GQ:PL	0/1:7,8:15:99:235,0,233
9	chrI	1421	.	G	A	196.85	.	AC=1;AF=0.50;AN=2;BaseQRankSum=-1.096;DP=1	GT:AD:DP:GQ:PL	0/1:7,8:15:99:227,0,228

25.3) ***Set of uncovered regions (***BED file***)*** (*BEDtools*). (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat)

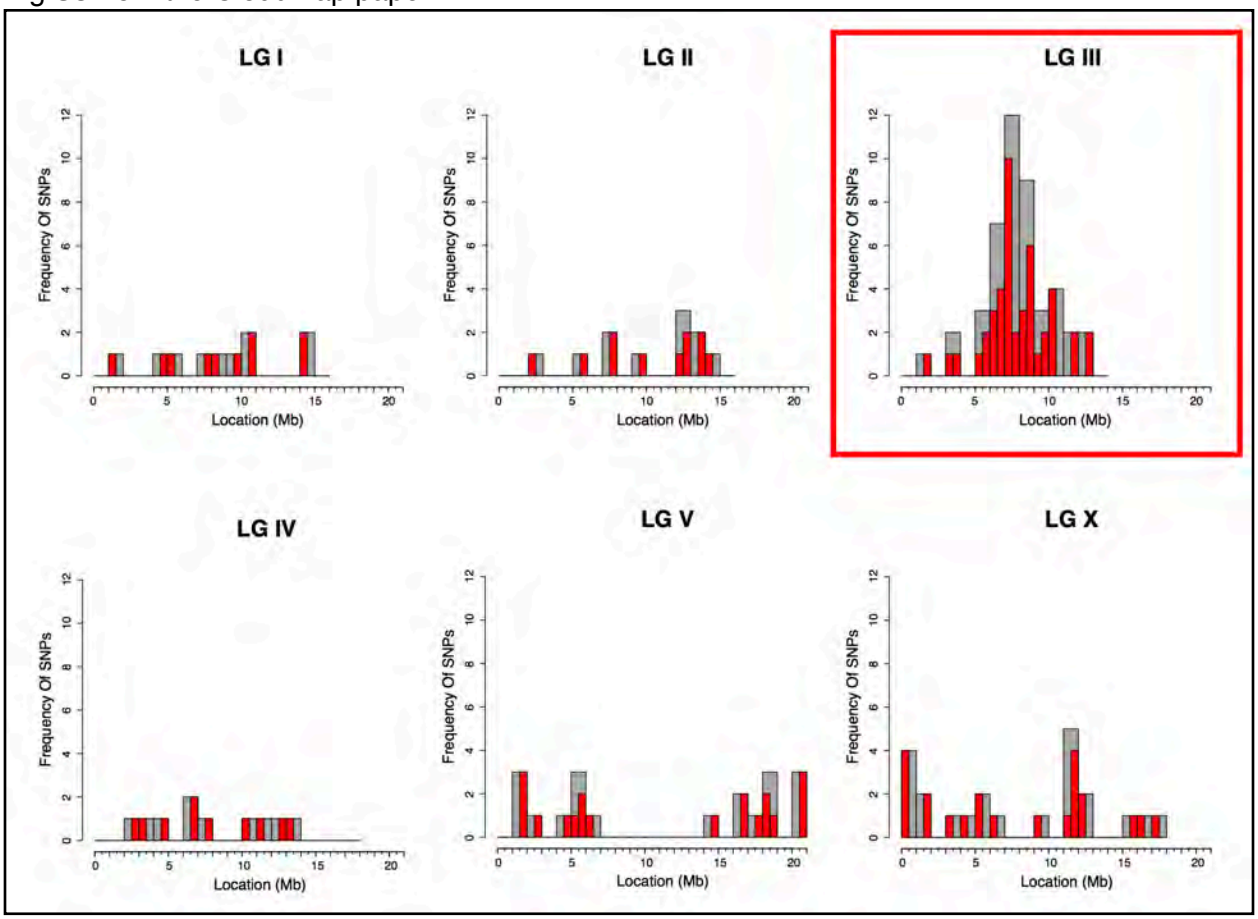
	A	B	C	D
1	chrI	2645	2664	0
2	chrI	3467	3482	0
3	chrI	3925	4014	0
4	chrI	8673	8703	0
5	chrI	8835	8995	0
6	chrI	9774	9787	0
7	chrI	11219	11317	0
8	chrI	11450	11469	0
9	chrI	15107	15117	0
10	chrI	15635	15767	0

Note: We strongly suggest that users employ the ***Subtract Variants*** and ***Uncovered Region Subtraction*** workflows if additional strains are available for this purpose. The general concept is shown in **Fig.5** of the CloudMap paper.

CloudMap EMS Variant Density Mapping Workflow

The ***EMS Variant Density Mapping*** workflow consists of the ***Unmapped Mutant*** workflow followed by the ***Subtract Variants*** workflow. The final VCF output is then plotted using the CloudMap ***EMS Variant Density Mapping*** tool. Readers are directed to the sections of this user guide that describe these workflows.

Fig.S3 from the CloudMap paper:



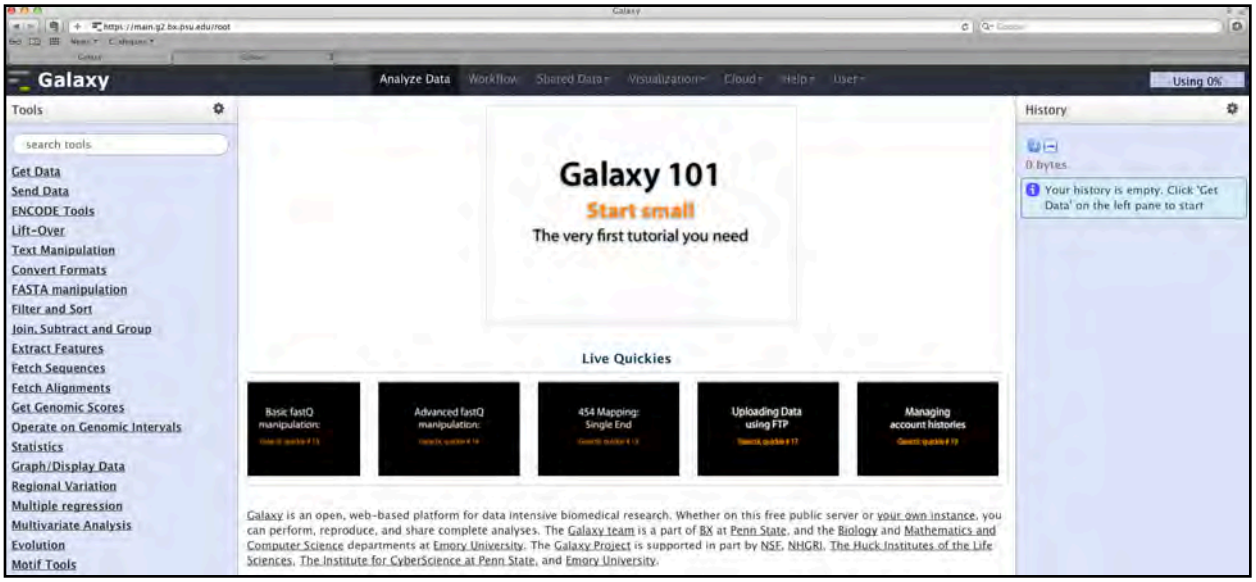
CloudMap | Cloud-based Pipeline for Analysis of Mutant Genome Sequences

CloudMap Subtract Variants workflow (using *ot266* Proof of Principle example from the CloudMap paper). A video version of this user guide is available at: http://usegalaxy.org/cloudmap.

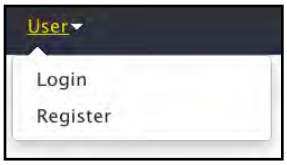
This workflow should be used downstream of either of the following workflows: ***Hawaiian Variant Mapping with WGS data and Variant Calling , EMS Density Mapping, or Unmapped Mutant workflows.*** Here we demonstrate the workflow using the *ot266* example from the Cloudmap paper (**Fig.8**). Users may apply this workflow to their own data by substituting the datasets in this example with their own datasets.

These workflows provide default function parameters, ensuring that users follow best practices, and allow for automated execution of sequential operations. We provide these workflows as helpful guides, but experienced users may execute functions in any meaningful order they please and may also create and share their own workflows to take advantage of the automation feature. More CloudMap documentation is available at http://usegalaxy.org/cloudmap.

1) Navigate to http://usegalaxy.org

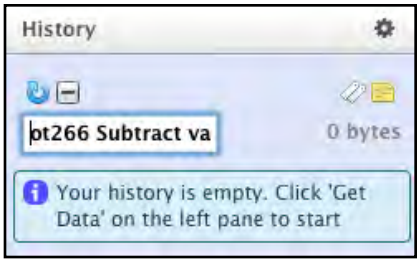


2) You should already have a Galaxy account at this point because you have run earlier workflows:

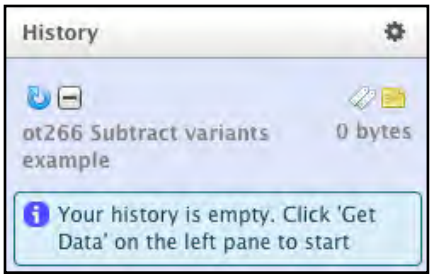


3) Once you are logged in using your email address, create a new history:

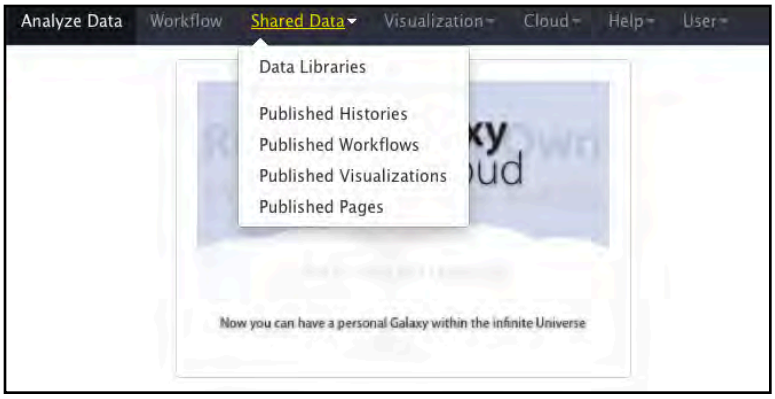
4) Now name that history “ot266 Subtract variants example”:



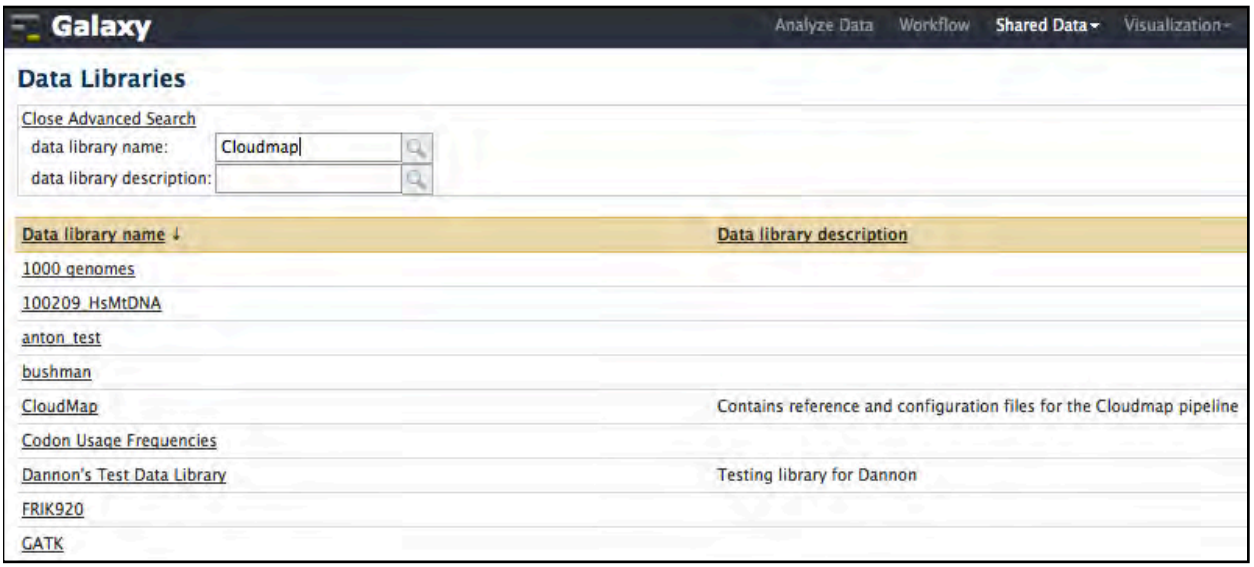
5) You now need to import the ***ot266 Proof of principle*** files or your own files to run the workflow:



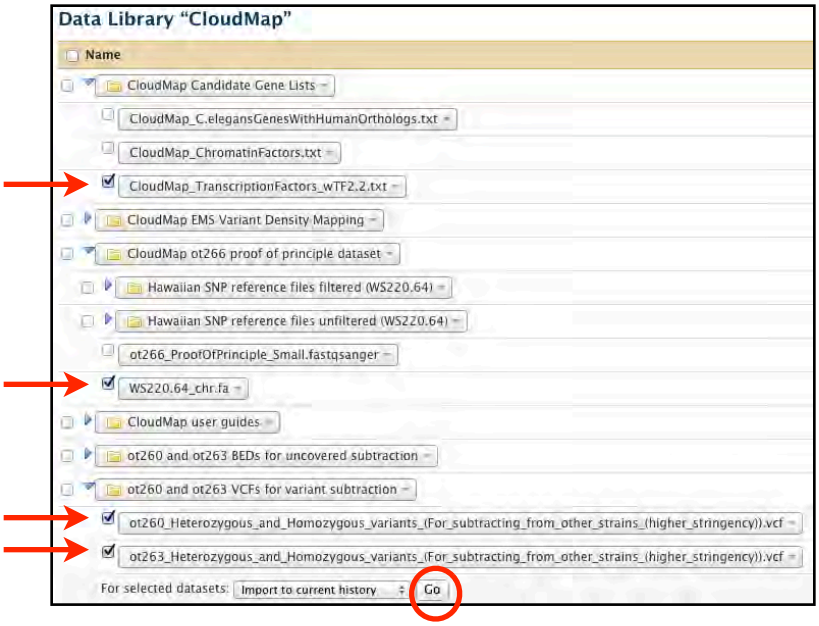
6) Click on the ***Shared Data*** link at the top of the page:



7) Click on ***Data Libraries*** to view the CloudMap data library:



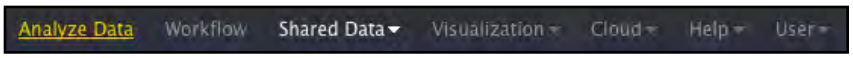
8) Click on the ***CloudMap*** library and select the 4 data files below for the *ot266* example. Then click "Go" to import these files into your history.



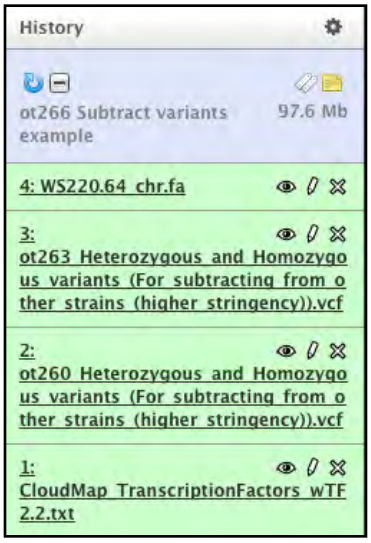
In an effort to subtract as many variants as possible, we subtract not only homozygous variants from other strains, but also heterozygous variants (*ot260* and *ot263* in this example). Such a strategy assumes that phenotype-inducing homozygous mutant variants in the strain under analysis are unlikely to be heterozygous in strains that will be used for subtraction. It is especially important to apply this strategy when subtracting variant lists generated using the *Hawaiian Variant Mapping with WGS Data* approach (see section "**CloudMap *Hawaiian Variant Mapping with WGS Data*** tool"), since background variants will be present in a heterozygous state in these pooled samples as a consequence of the mapping cross. We also subtract Hawaiian SNPs in this workflow.

9) You will see that the files have been imported successfully:

10) Click on ***Analyze Data*** to see the files in your history:

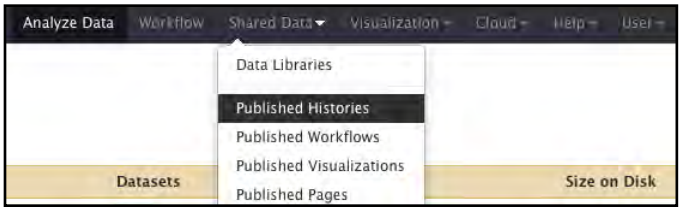


11) You will now see these files in your history:

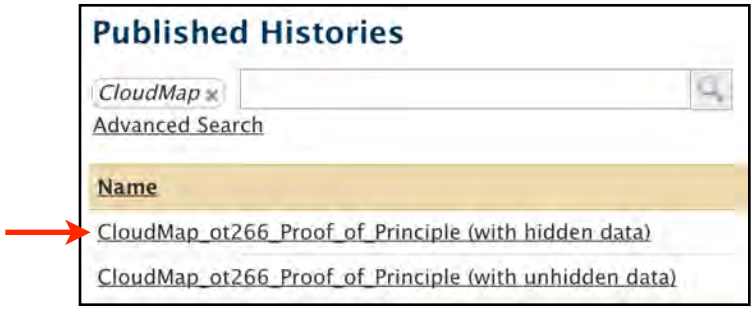


12) You will also need to import homozygous variants (VCF file) from the workflow you performed earlier. In this example, we will use the *ot266* homozygous variants from running the ***Hawaiian Variant Mapping with WGS Data and Variant Calling*** workflow. The *ot266* example history is shared so we will import the homozygous variants from that history. Note: the *ot260* and *ot263* variants that we use for data subtraction in this example come from strains that were not mapped with Hawaiian, while the *ot266* sample was mapped with Hawaiian.

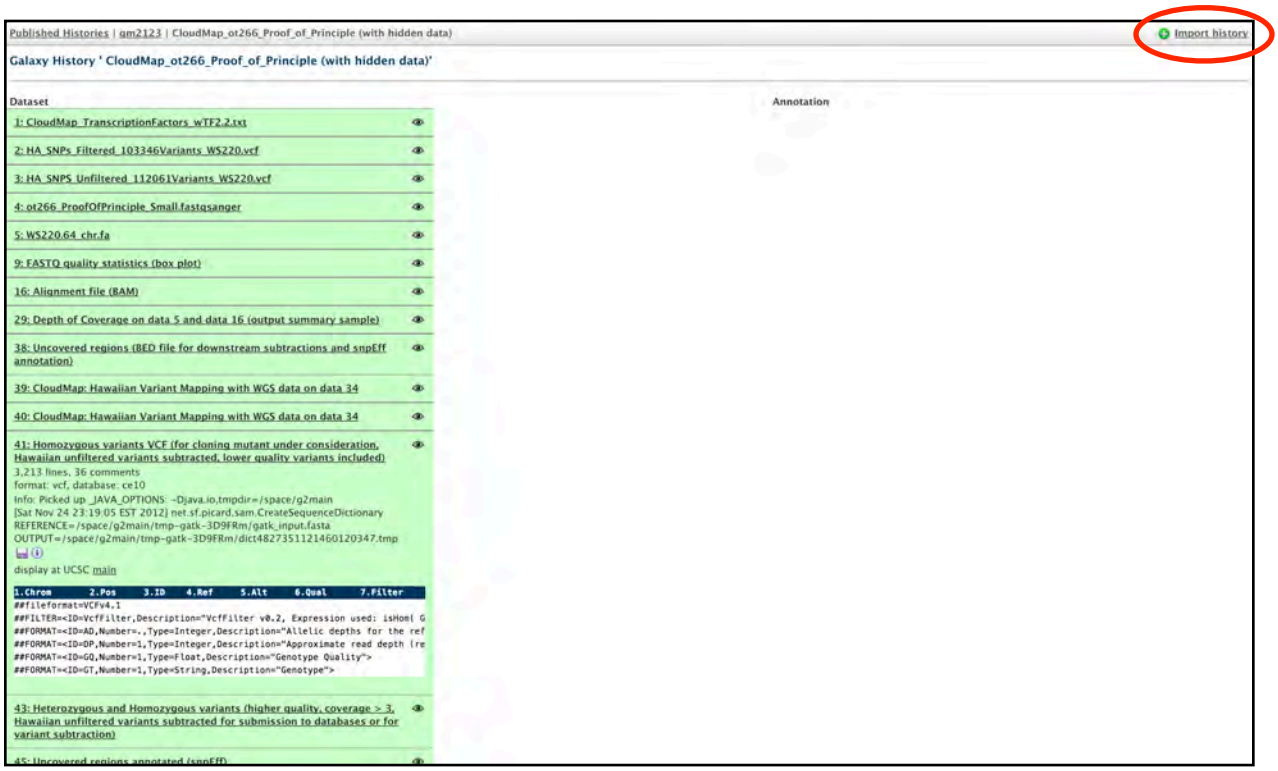
Click on ***Shared Data***—> ***Published Histories***:



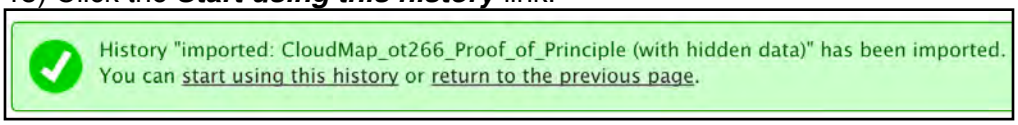
13) Click on the history ***CloudMap: ot266 Proof of Principle (with hidden data)***:



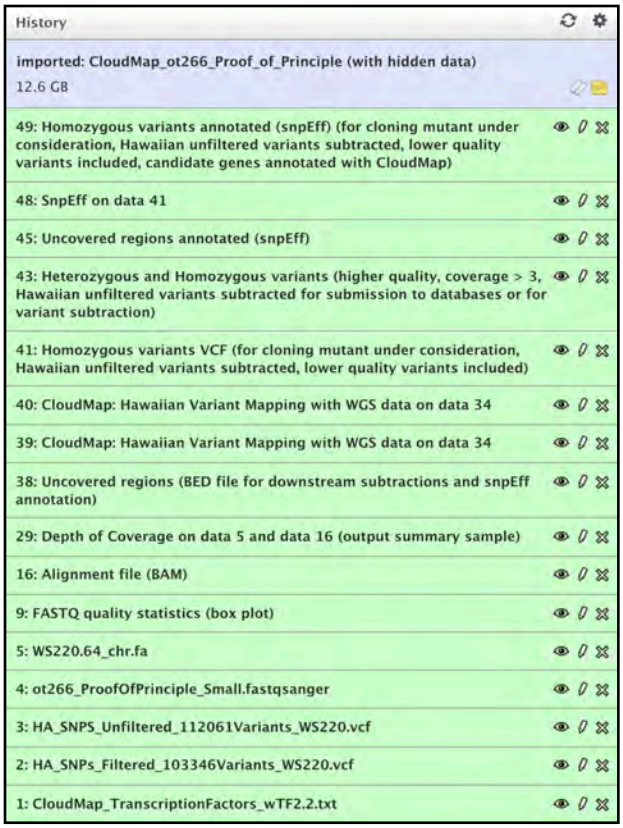
14) Import the *ot266* history. The homozygous variants VCF we will subtract ot260 and ot263 variants from is expanded in this screenshot.



15) Click the ***Start using this history*** link.

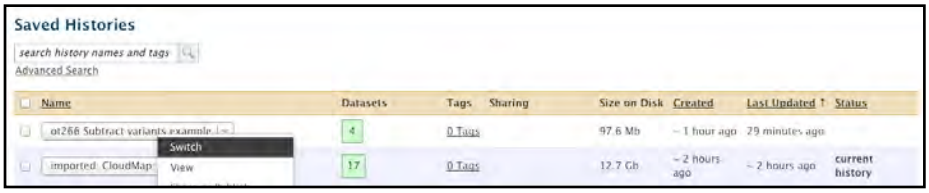


16) You now can view all the files in the *ot266* history.

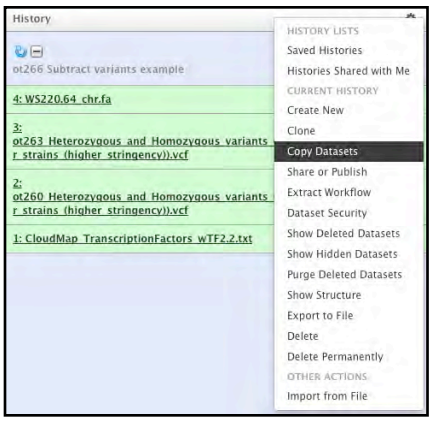


17) Switch back to the ***ot266 Subtract Variants example*** history you created earlier by clicking ***Saved HIstories*** in your history options.

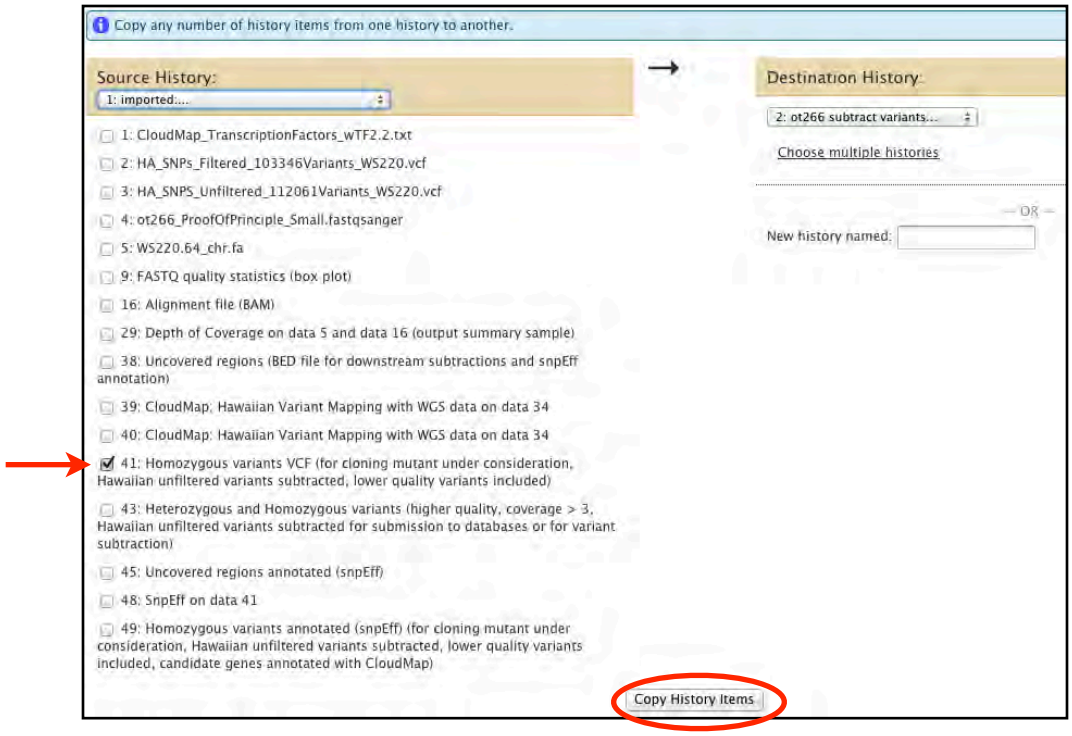
18) Click on the ***ot266 Subtract Variants example*** history and click ***Switch*** to return to that history:



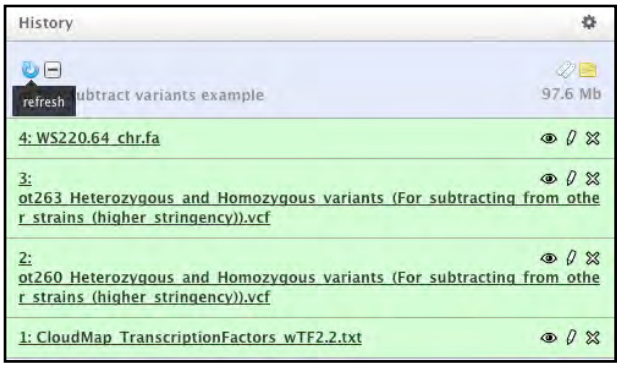
19) To copy the *ot266* homozygous variants into this history, click ***Copy Datasets*** in your history options:



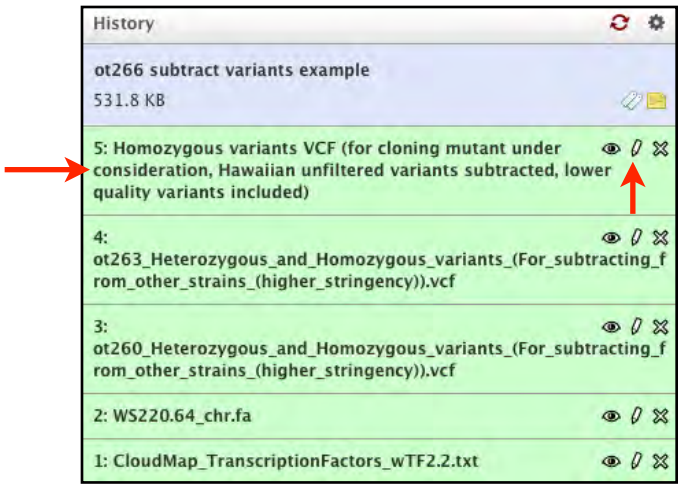
20) Copy the *ot266* ***Homozygous variants VCF*** from the newly imported *ot266* history:



21) Hit refresh in your history:

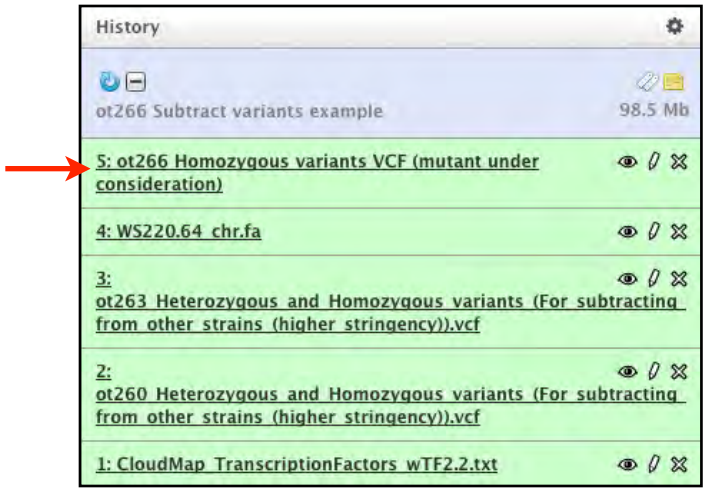


22) You will now see the ***ot266 Homozygous Variants*** (VCF) in your history. Click on the pencil icon to change the name of the file to add the *ot266* prefix.

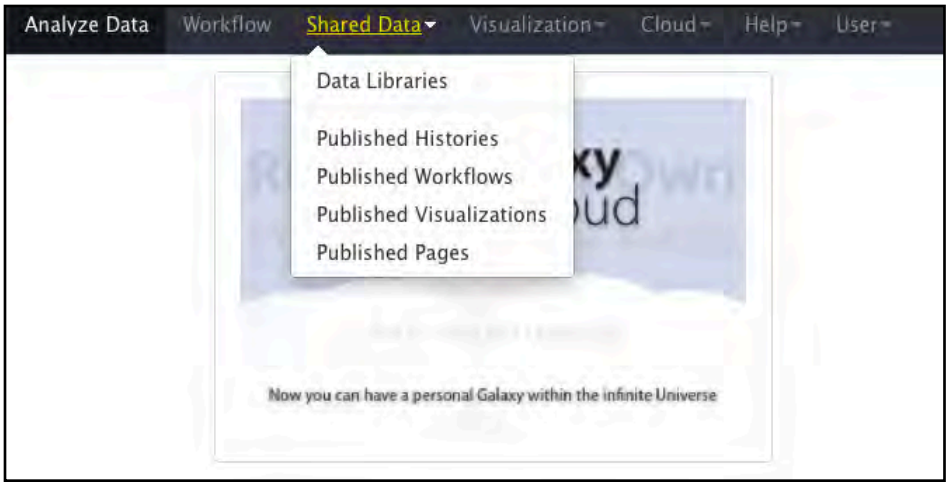


23) Add the *ot266* prefix to the file name:

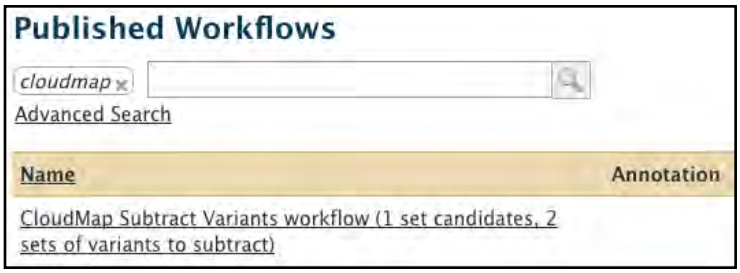
24) You will see that the file name has been updated:



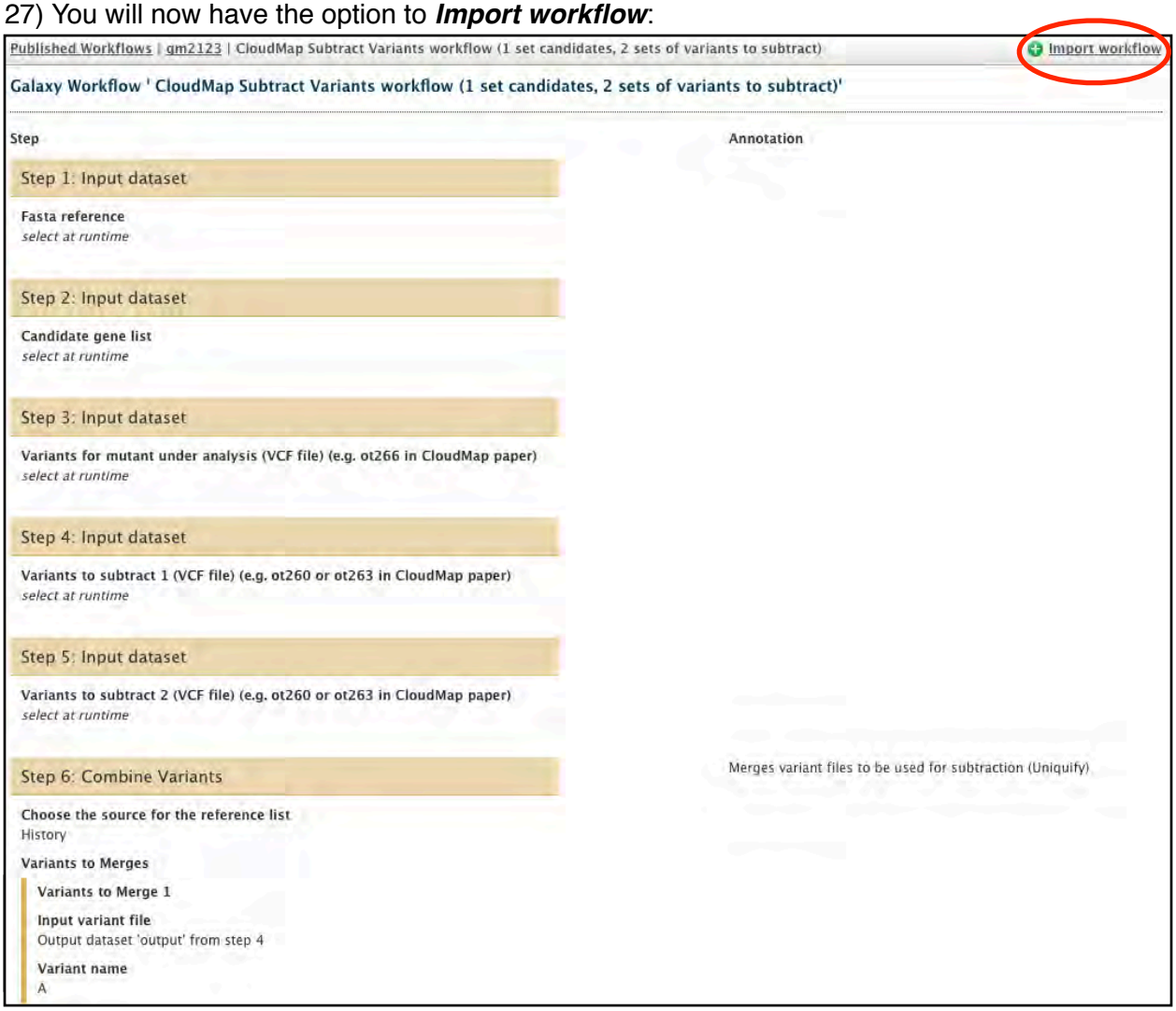
25) Now you have all the files ready to run the ***Subtract Variants*** workflow. Click on the ***Shared Data—>Published Workflows*** link at the top of the page:



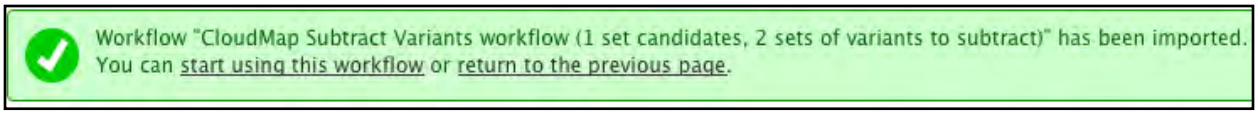
26) Select the ***CloudMap Subtract Variants*** workflow:



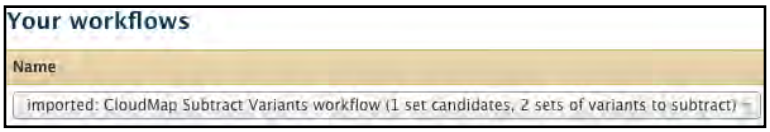
27) You will now have the option to ***Import workflow***:



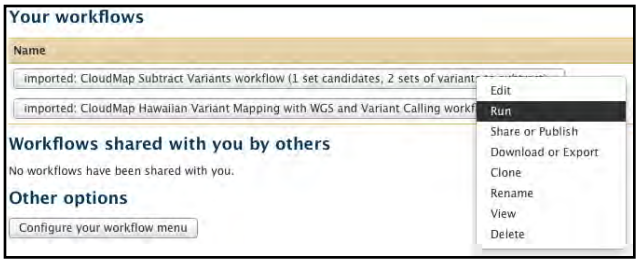
28) You will see a message indicating that the workflow has been imported:



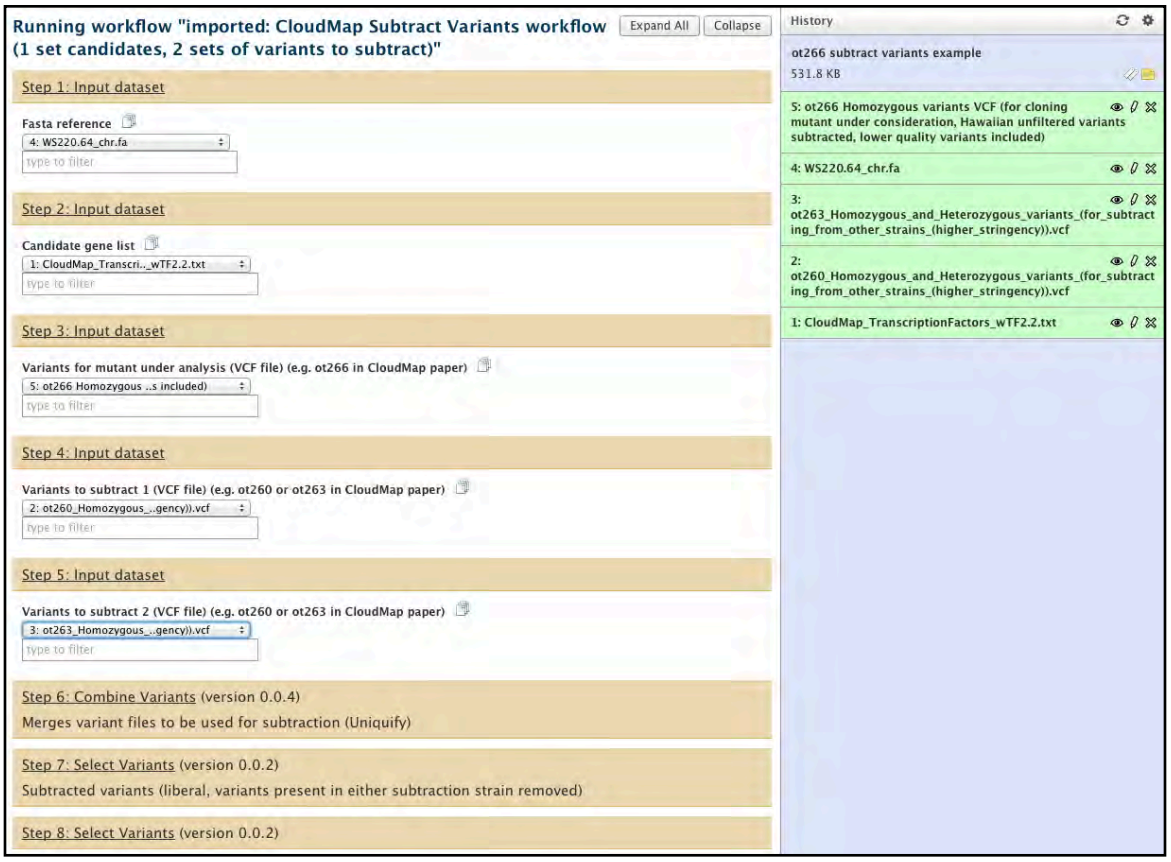
29) Click ***Start using this workflow*** and you will see that the workflow has been imported. From now on, you can easily access this workflow under the ***Workflow*** tab.



30) Click on the workflow and select ***Run***:

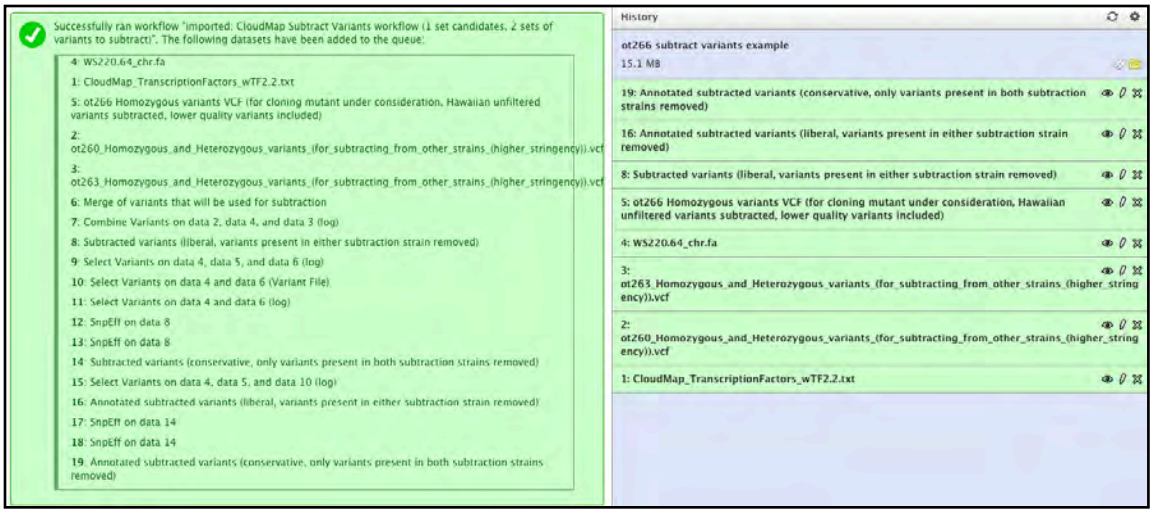


31) You will see all the steps in the workflow prior to running it. Make sure that each of the input fields corresponds to the appropriate file in your history. Click ***Run Workflow*** when ready.

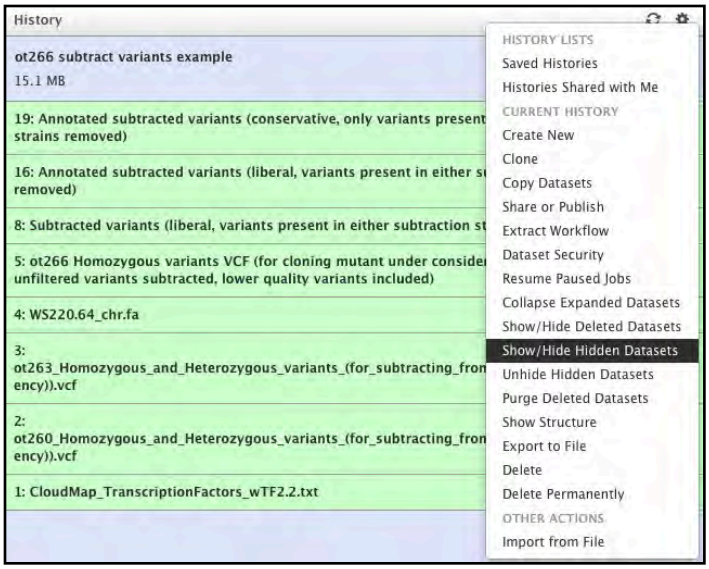


32) All of the automated functions have the appropriate default parameters configured, although experienced users may want to modify these prior to running. Once you are ready to run the workflow, press ***Run Workflow*** and the workflow will start (this step takes a minute or two to begin, be patient and don't hit the ***Run Workflow*** button repeatedly). You will receive an email when the workflow is completed:

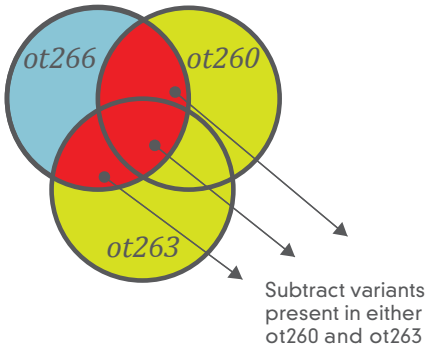
33) The workflow has finished running and you can view the resulting output:



34) You will notice that while approximately 20 output files were generated during the course of the workflow (output files are sequentially numbered), only some output files remain visible while others are hidden. The visible files are most important for analysis of the mutant under consideration or downstream analysis. In order to view hidden files, click ***Show Hidden Datasets*** in the History menu:



35) There are 3 main output files. The first, named ***Subtracted variants (liberal, variants present in either subtraction strain removed)*** is a VCF file generated by *GATK* that corresponds to the variant subtraction described in **Fig.8** of the CloudMap paper.



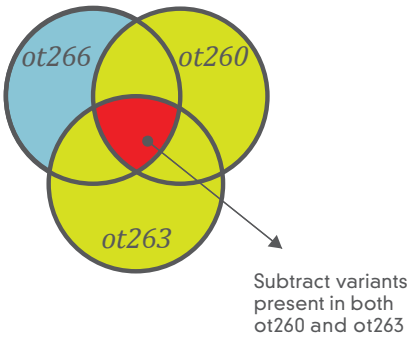
This file contains *ot266* homozygous variants after both homozygous and heterozygous variants present in **either** *ot260* **or** *ot263* have been subtracted. This file should be downloaded to be easily viewed in its entirety. The first several lines in any VCF file are header lines starting with "#" so users who wish to filter or sort these files in Excel are advised to remove the header lines. (For more information on file format, see: http://genome.ucsc.edu/FAQ/FAQformat.html). Below you can see a snippet of the file after header lines have been removed:

	A	B	C	D	E	F	G	H	I	J
1	#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	rgSM
2	chrI	62642	.	T	C	48.77	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.02:80,6,0
3	chrI	346149	.	T	A	85.77	PASS	AC=2;AF=1.00;AN=2;DP=3;	GT:AD:DP:GQ:PL	1/1:0,3:3:9.03:118,9,0
4	chrI	369870	.	C	T	48.08	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.02:79,6,0
5	chrI	369871	.	C	T	48.77	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.02:80,6,0
6	chrI	663697	.	G	C	167.29	PASS	AC=2;AF=1.00;AN=2;DP=5;	GT:AD:DP:GQ:PL	1/1:0,5:5:15.05:200,15,0
7	chrI	670146	.	G	A	36.43	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.01:68,6,0
8	chrI	670173	.	T	C	36.43	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.01:68,6,0
9	chrI	671425	.	T	A	48.77	PASS	AC=2;AF=1.00;AN=2;DP=2;	GT:AD:DP:GQ:PL	1/1:0,2:2:6.02:80,6,0
10	chrI	687402	.	T	A	67.01	PASS	AC=2;AF=1.00;AN=2;DP=3;	GT:AD:DP:GQ:PL	1/1:0,3:3:9.01:99,9,0
11	chrI	714649	.	C	G	67.78	PASS	AC=2;AF=1.00;AN=2;DP=3;	GT:AD:DP:GQ:PL	1/1:0,3:3:9.02:100,9,0

36) The file ***Annotated subtracted variants (liberal, variants present in either subtraction strain removed)*** is simply the VCF described in the previous step which has now had its variants annotated for their predicted effect on genes with *snpEff*. The ***CloudMap Candidate Checker*** has also annotated any candidate genes that appear in the *snpEff* output.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	# Chrom	Position	Reference	Change	Change_type	Homozygous	Quality	Coverage	Warnings	Gene_ID	Gene_name	Bio_type	Transcript_ID	Exon_ID	Exon_Rank	Effect	old_AA/new_	Old_codon/N	Codon_Num	Codon_Degen	CDS_size
2	I	62642	T	C	SNP	Hom	48.77	2		Y48G1C.4	pgs-1	protein_codi	Y48G1C.4			DOWNSTREAM: 8216 bases					
3	I	62642	T	C	SNP	Hom	48.77	2		Y48G1C.5	Y48G1C.5	protein_codi	Y48G1C.5			INTRON					3486
4	I	62642	T	C	SNP	Hom	48.77	2		Y48G1C.2	csk-1	protein_codi	Y48G1C.2.1			UPSTREAM: 9218 bases					
5	I	62642	T	C	SNP	Hom	48.77	2		Y48G1C.2	csk-1	protein_codi	Y48G1C.2.2			UPSTREAM: 9236 bases					
6	I	62642	T	C	SNP	Hom	48.77	2		Y48G1C.2	csk-1	protein_codi	Y48G1C.2.3			UPSTREAM: 9869 bases					
7	I	346149	T	A	SNP	Hom	85.77	3		Y48G1A.3	Y48G1A.3	protein_codi	Y48G1A.3			DOWNSTREAM: 8304 bases					
8	I	346149	T	A	SNP	Hom	85.77	3		Y48G1A.1	Y48G1A.1	protein_codi	Y48G1A.1			UPSTREAM: 2389 bases					
9	I	346149	T	A	SNP	Hom	85.77	3		Y48G1A.6	mbtr-1	protein_codi	Y48G1A.6b			INTRON					1636
10	I	346149	T	A	SNP	Hom	85.77	3		Y48G1A.6	mbtr-1	protein_codi	Y48G1A.6a			INTRON					1695
11	I	346149	T	A	SNP	Hom	85.77	3		Y48G1A.2	Y48G1A.2	protein_codi	Y48G1A.2.2			UPSTREAM: 1316 bases					
12	I	346149	T	A	SNP	Hom	85.77	3		Y48G1A.2	Y48G1A.2	protein_codi	Y48G1A.2.1			UPSTREAM: 1323 bases					
13	I	369870	C	T	SNP	Hom	48.08	2		R119.3	R119.3	protein_codi	R119.3.1			DOWNSTREAM: 3480 bases					
14	I	369870	C	T	SNP	Hom	48.08	2		R119.3	R119.3	protein_codi	R119.3.2			DOWNSTREAM: 3704 bases					
15	I	369870	C	T	SNP	Hom	48.08	2		R119.1	R119.1	protein_codi	R119.1			UPSTREAM: 5966 bases					
16	I	369870	C	T	SNP	Hom	48.08	2		R119.4	pqn-59	protein_codi	R119.4.1			DOWNSTREAM: 7608 bases					
17	I	369870	C	T	SNP	Hom	48.08	2		R119.2	R119.2	protein_codi	R119.2			INTRON					1089
18	I	369870	C	T	SNP	Hom	48.08	2		R119.7	rnp-8	protein_codi	R119.7			DOWNSTREAM: 1359 bases					
19	I	369870	C	T	SNP	Hom	48.08	2		R119.4	pqn-59	protein_codi	R119.4.2			DOWNSTREAM: 9377 bases					
20	I	369871	C	T	SNP	Hom	48.77	2		R119.3	R119.3	protein_codi	R119.3.1			DOWNSTREAM: 3479 bases					
21	I	369871	C	T	SNP	Hom	48.77	2		R119.3	R119.3	protein_codi	R119.3.2			DOWNSTREAM: 3703 bases					
22	I	369871	C	T	SNP	Hom	48.77	2		R119.1	R119.1	protein_codi	R119.1			UPSTREAM: 5967 bases					
23	I	369871	C	T	SNP	Hom	48.77	2		R119.4	pqn-59	protein_codi	R119.4.1			DOWNSTREAM: 7607 bases					
24	I	369871	C	T	SNP	Hom	48.77	2		R119.2	R119.2	protein_codi	R119.2			INTRON					1089

37) The final file, ***Annotated subtracted variants (conservative, only variants present in both subtraction strains removed)*** is exactly the same as the file in step #36 with the only exception being that only variants present in **both** *ot260* and *ot263* were subtracted from *ot266*. We label this file "conservative" because it is less likely that a causal variant in ot266 will be incorrectly subtracted since that same causal variant would have to be present in **both** *ot260* and *ot263*.



Note: We strongly suggest that users employ the ***Uncovered Region Subtraction*** workflow using the same strains (from their own screens) used in this workflow for variant subtraction. The general concept is shown in **Fig.5** of the CloudMap paper and is the same as used in this ***Subtract Variants*** workflow.

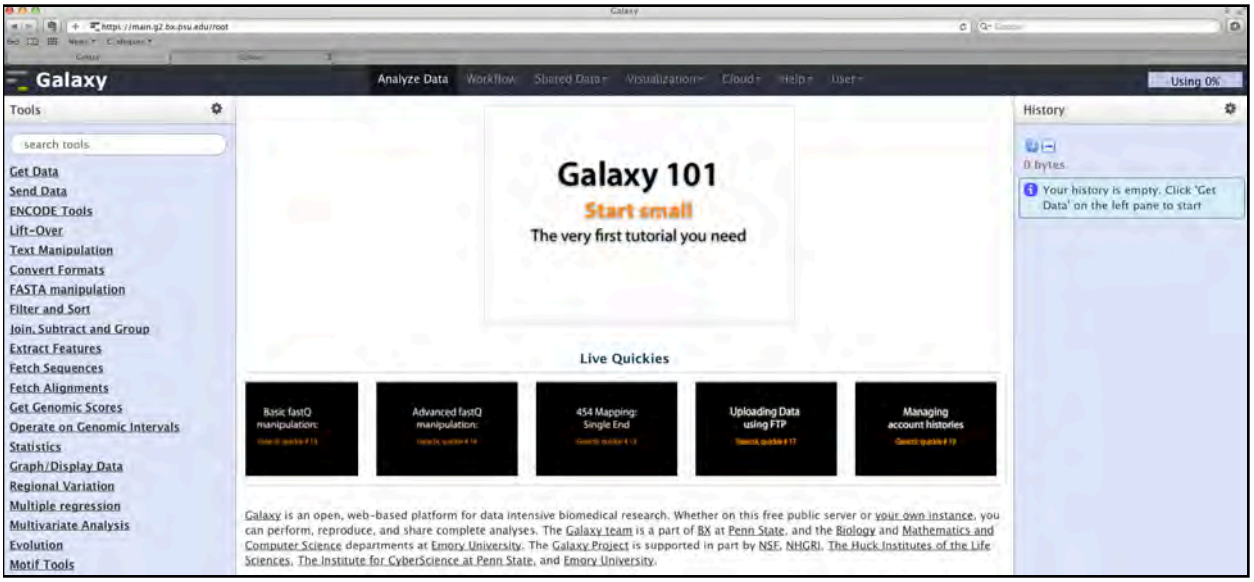
Also, please note that the number of variants per sample in this example do not match that in **Fig.8** of the CloudMap paper because the ot266 dataset used is a small subset of the full FASTQ file for that sample.

CloudMap | Cloud-based Pipeline for Analysis of Mutant Genome Sequences

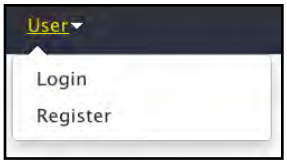
CloudMap Uncovered Region Subtraction workflow (using *ot266* Proof of Principle example from the CloudMap paper). A video version of this user guide is available at: http://usegalaxy.org/cloudmap. This workflow should be used downstream of either of the following workflows: ***Hawaiian Variant Mapping with WGS data and Variant Calling , EMS Density Mapping, or Unmapped Mutant workflows.*** Here we demonstrate the workflow using the *ot266* example from the Cloudmap paper (**Fig.8**). The goal is to subtract uncovered regions present in both *ot260* and *ot263* from uncovered regions in *ot266* (all from the same starting strain) and then to annotate the resulting uncovered regions for whether they intersect with functional genomic units (genes, ncRNAs, etc). Users may apply this workflow to their own data by substituting the datasets in this example with their own datasets.

These workflows provide default function parameters, ensuring that users follow best practices, and allow for automated execution of sequential operations. We provide these workflows as helpful guides, but experienced users may execute functions in any meaningful order they please and may also create and share their own workflows to take advantage of the automation feature. More CloudMap documentation is available at http://usegalaxy.org/cloudmap.

1) Navigate to http://usegalaxy.org

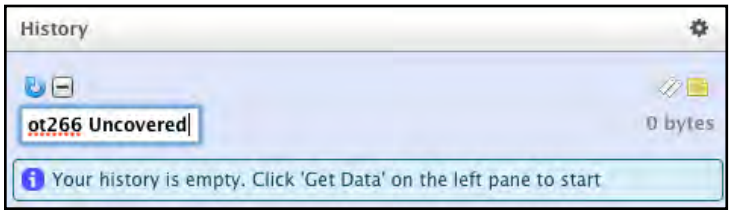


2) You should already have a Galaxy account at this point because you have run earlier workflows:

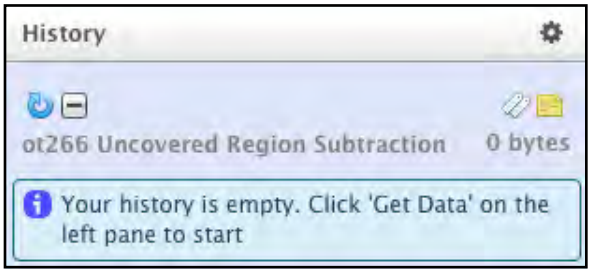


3) Once you are logged in using your email address, create a new history:

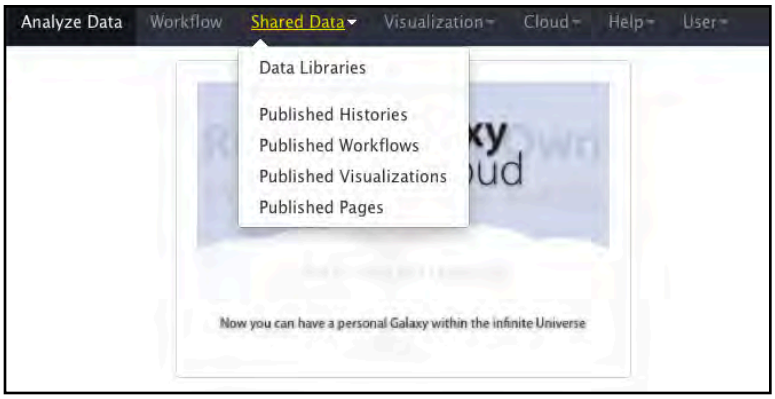
4) Now name that history:



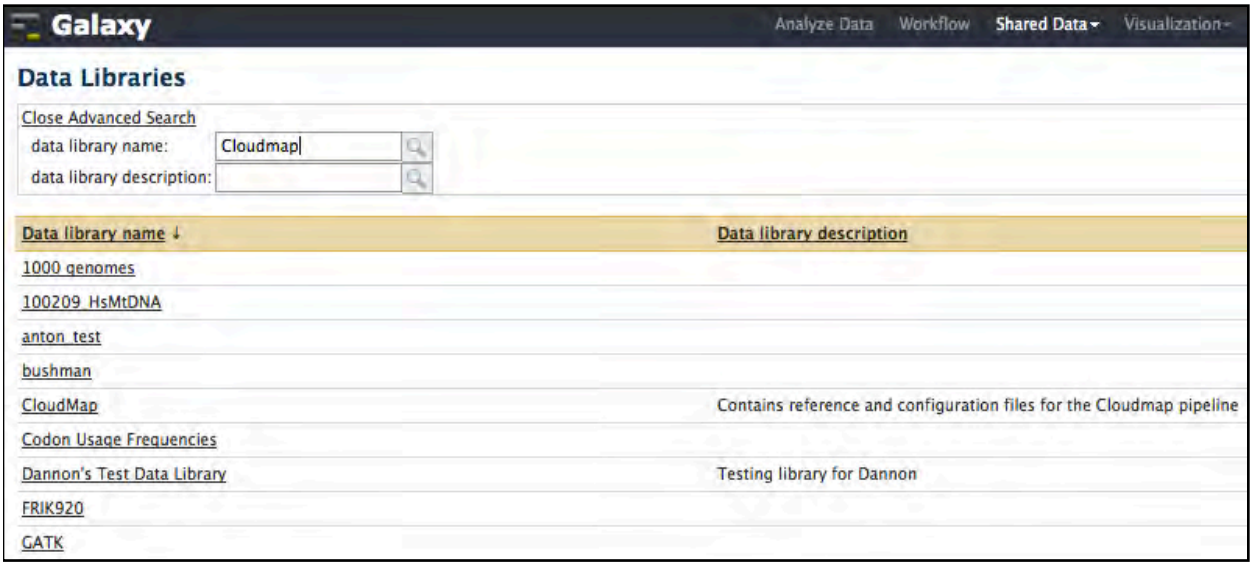
5) The history has been renamed.



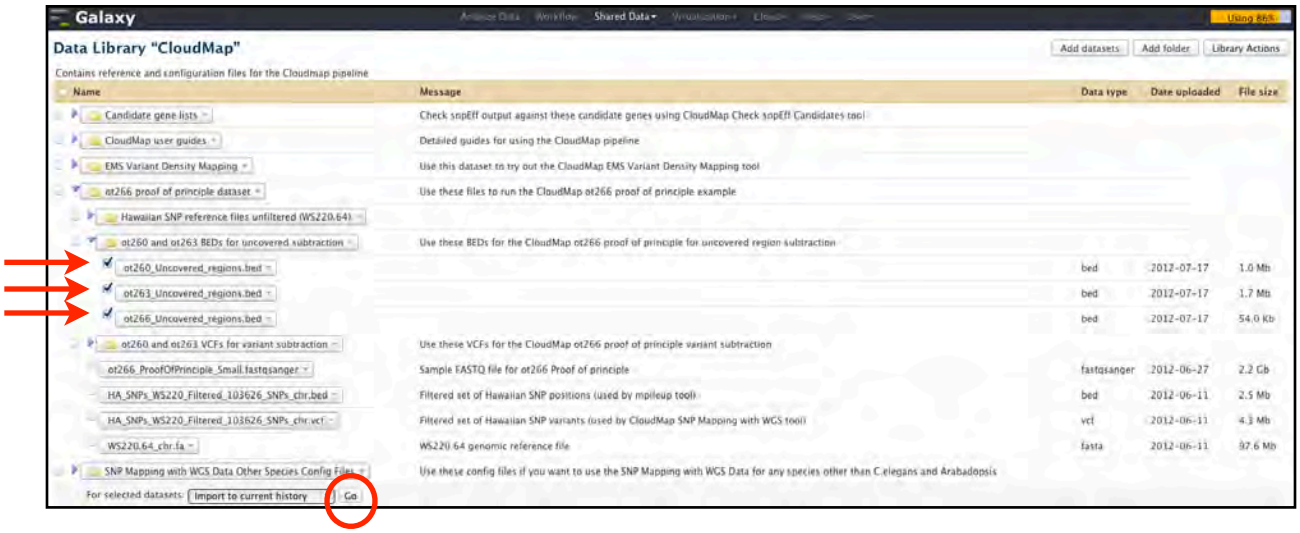
6) You now need to import the ***ot266 Proof of principle*** files (from the CloudMap Shared Data library) or your own files to run the workflow (***See the Analyze Your Own Data Using CloudMap Workflows*** section of this user guide).



7) Click on ***Data Libraries*** to view the CloudMap data library:

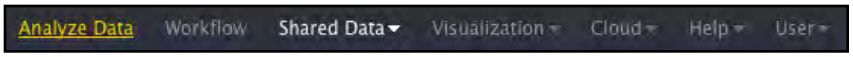


8) Click on the ***CloudMap*** library and select the 3 data files below for the *ot266* example. Then click “Go” to import these files into your history.



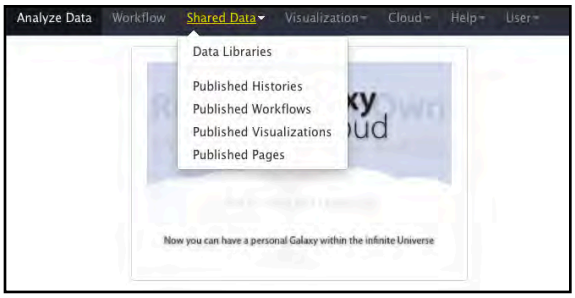
9) You will see that the files have been imported successfully:

10) Click on ***Analyze Data*** to see the files in your history:

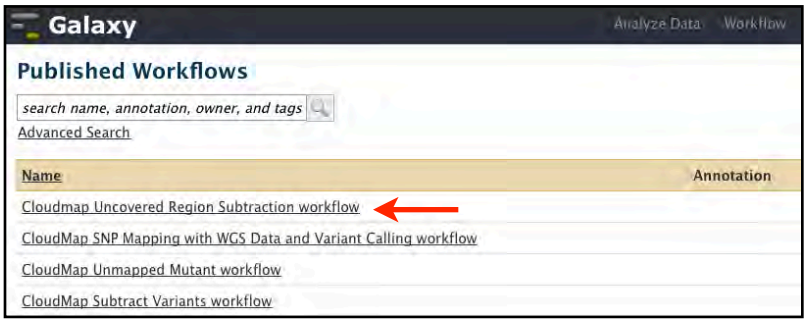


11) You will now see these files in your history:

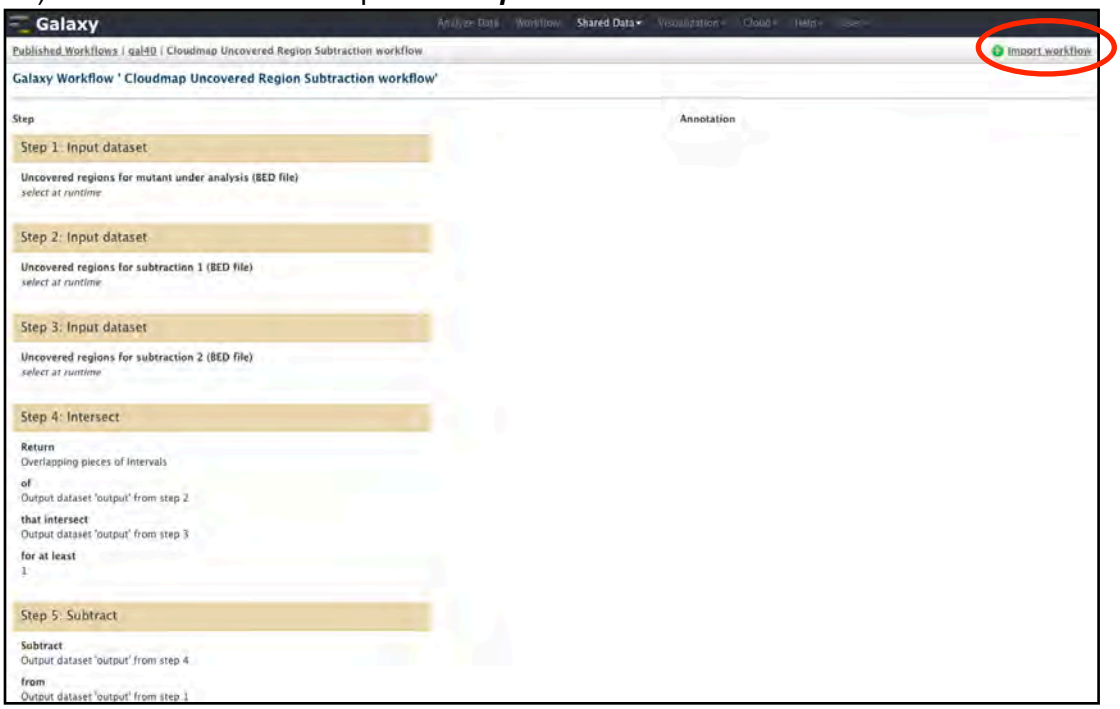
12) Now you have all the files ready to run the ***Uncovered Region Subtraction*** workflow. Click on the ***Shared Data—>Published Workflows*** link at the top of the page:



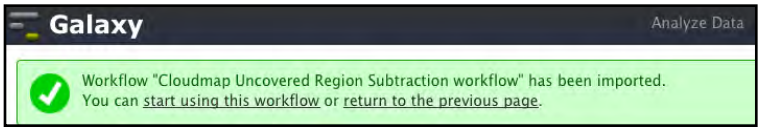
13) Select the ***Uncovered Region Subtraction*** workflow:



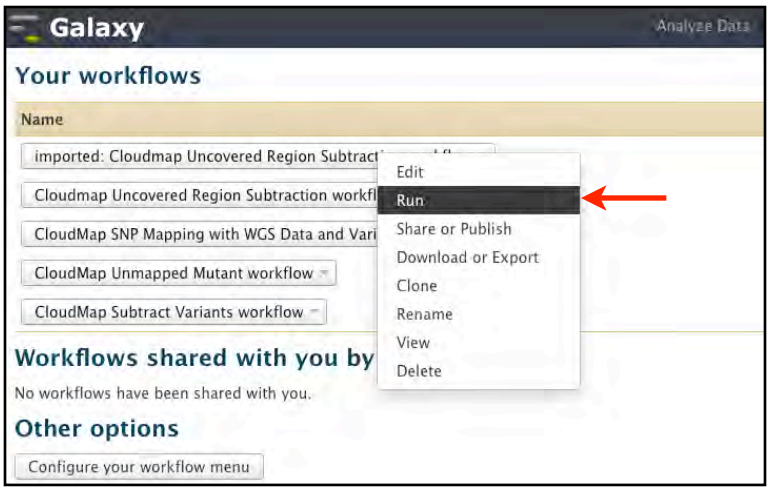
14) You will now have the option to ***Import workflow***:



15) You will see a message indicating that the workflow has been imported:

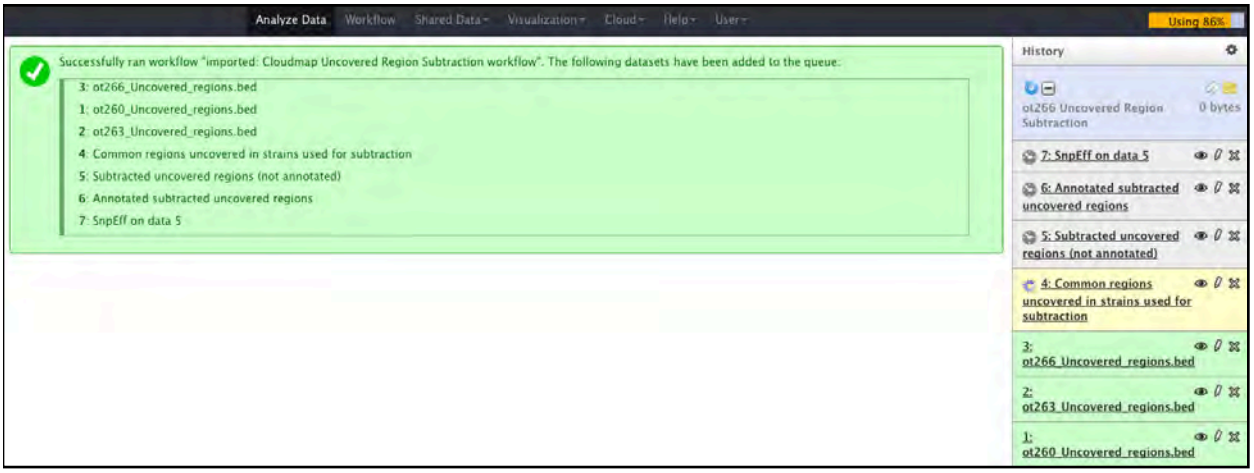


16) Click ***Start using this workflow*** and you will see that the workflow has been imported. From now on, you can easily access this workflow under the ***Workflow*** tab. Click on the workflow and select ***Run***:

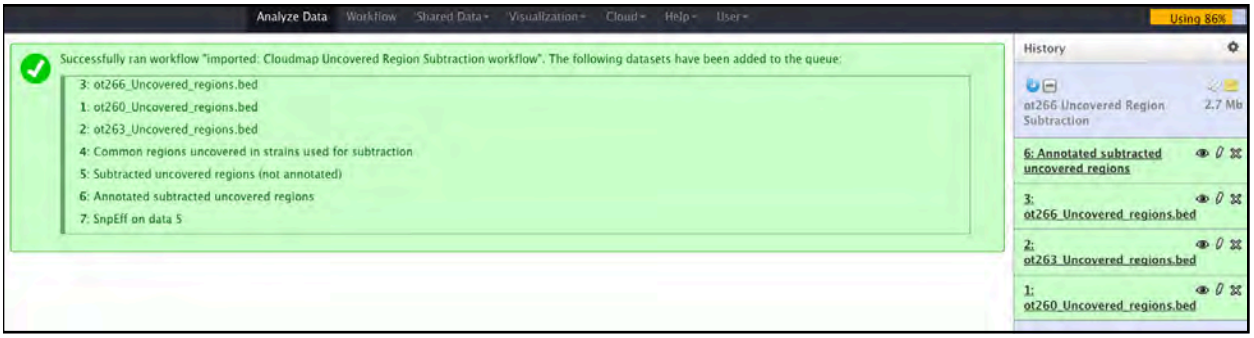


17) You will see all the steps in the workflow prior to running it. Make sure that each of the input fields corresponds to the appropriate file in your history. In our example, we want to subtract uncovered regions present in both *ot260* and *ot263* from the uncovered regions in *ot266*. Click ***Run Workflow*** when ready.

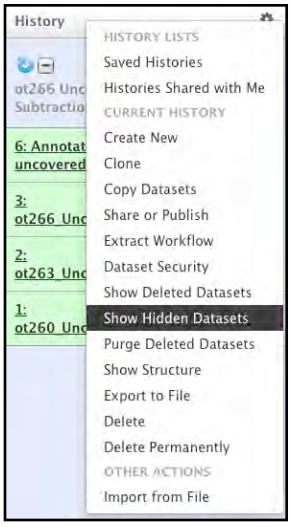
18) All of the automated functions have the appropriate default parameters configured, although experienced users may want to modify these prior to running. Once you are ready to run the workflow, press ***Run Workflow*** and the workflow will start (this step takes a minute or two to begin, be patient and don't hit the ***Run Workflow*** button repeatedly). You will receive an email when the workflow is completed:



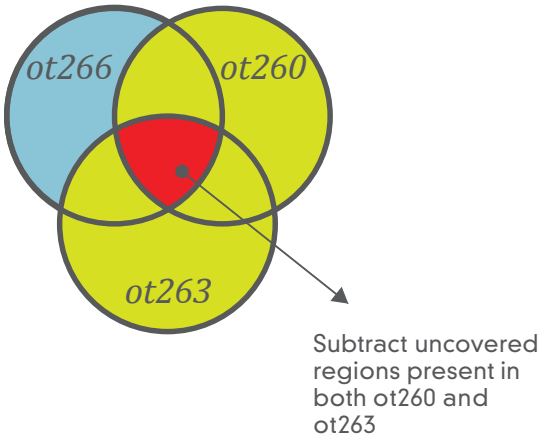
19) The workflow has finished running and you can view the resulting output:



20) You will notice that while 4 output files were generated during the course of the workflow (output files are sequentially numbered), only one output file remains visible while others are hidden. The one visible file (***Annotated subtracted uncovered regions***) is the most important for analysis of the mutant under consideration. In order to view hidden files, click ***Show Hidden Datasets*** in the History menu:



21) The ***Annotated subtracted uncovered regions*** output file conceptually corresponds to the ***Annotated subtracted variants (conservative, only variants present in both subtraction strains removed)*** file generated by the ***Subtract Variants*** workflow. This conservative strategy, as shown below, aims to only subtract uncovered regions that are present in both *ot260* and *ot263.* By selecting uncovered regions that only appear in more than one sample, we hope to err on the side of subtracting true deletions as opposed to subtracting regions that are simply uncovered in a given sample.



22) The ***Annotated subtracted uncovered regions*** output file (snpEff) is shown below:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	# Chromo	Position	Reference	Change	Change_type	Homozygous	Quality	Coverage	Warnings	Gene_ID	Gene_name	Bio_type	Transcript_ID	Exon_ID	Exon_Rank	Effect	old_AA/new_AA
2	I	2646	2664			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.4			UPSTREAM: 8859 bases
3	I	2646	2664			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.6			UPSTREAM: 8972 bases
4	I	2646	2664			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.3			UPSTREAM: 7767 bases
5	I	2646	2664			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.2			UPSTREAM: 8849 bases
6	I	2646	2664			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.1			UPSTREAM: 8853 bases
7	I	2646	2664			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.5			UPSTREAM: 8853 bases
8	I	2646	2664			Interval		0	0		Y74C9A.3	Y74C9A.3	protein_codi	Y74C9A.3.1			DOWNSTREAM: 1473 bases
9	I	2646	2664			Interval		0	0		Y74C9A.3	Y74C9A.3	protein_codi	Y74C9A.3.2			DOWNSTREAM: 1575 bases
10	I	2646	2664			Interval		0	0		Y74C9A.6	Y74C9A.6	snoRNA	Y74C9A.6			DOWNSTREAM: 1101 bases
11	I	3468	3482			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.4			UPSTREAM: 8037 bases
12	I	3468	3482			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.6			UPSTREAM: 8150 bases
13	I	3468	3482			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.3			UPSTREAM: 6945 bases
14	I	3468	3482			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.2			UPSTREAM: 8027 bases
15	I	3468	3482			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.1			UPSTREAM: 8031 bases
16	I	3468	3482			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.5			UPSTREAM: 8031 bases
17	I	3468	3482			Interval		0	0		Y74C9A.3	Y74C9A.3	protein_codi	Y74C9A.3.1			DOWNSTREAM: 651 bases
18	I	3468	3482			Interval		0	0		Y74C9A.3	Y74C9A.3	protein_codi	Y74C9A.3.2			DOWNSTREAM: 753 bases
19	I	3468	3482			Interval		0	0		Y74C9A.6	Y74C9A.6	snoRNA	Y74C9A.6			DOWNSTREAM: 279 bases
20	I	3976	4014			Interval		0	0		Y74C9A.2	nlp-40	protein_codi	Y74C9A.2.4			UPSTREAM: 7579 bases

CloudMap | Cloud-based Pipeline for Analysis of Mutant Genome Sequences

Analyzing your own data with CloudMap and Galaxy:

The various sections of this user guide detail how to analyze sample datasets from the CloudMap paper. In order to analyze your own sequencing data (in the form of FASTQ files), a few quick steps need to be performed prior to running the workflows detailed in this user guide.

For more details, please see the CloudMap paper or visit the CloudMap website at: http://usegalaxy.org/cloudmap. Video versions of these user guides are also available at this website.

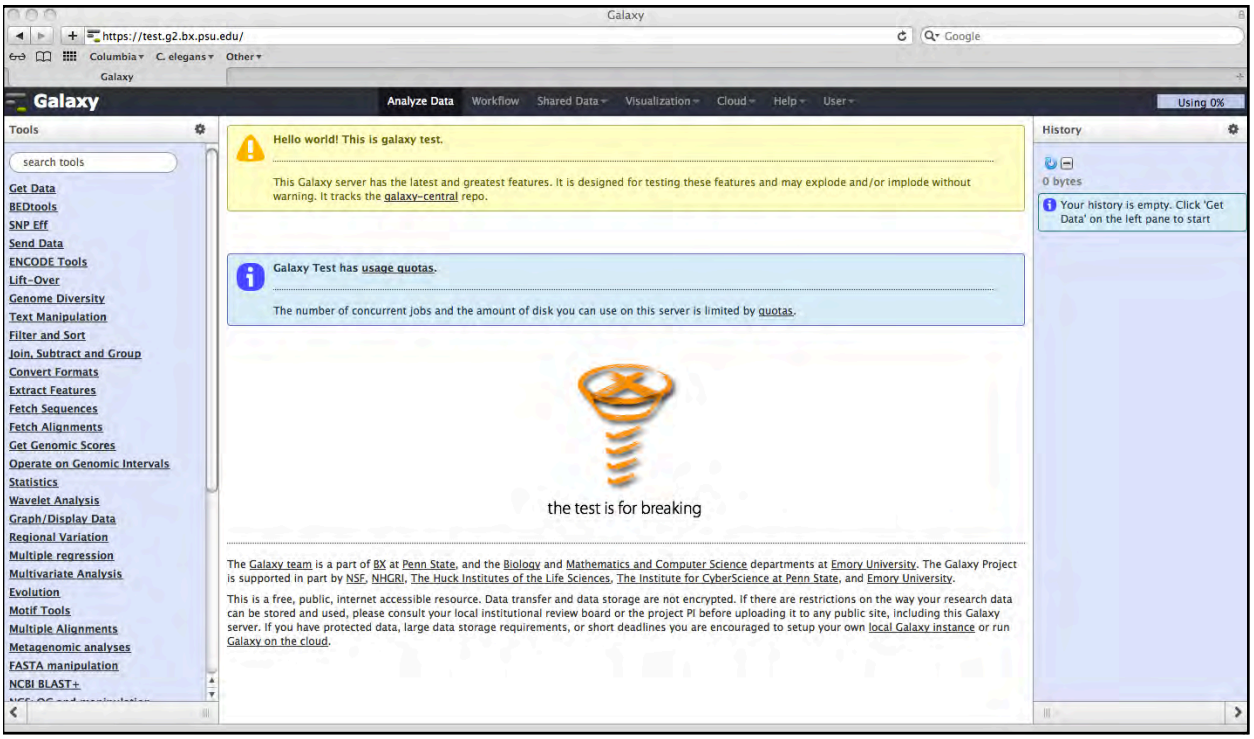
Useful Galaxy screencasts are available here: http://wiki.g2.bx.psu.edu/Learn/Screencasts

SECTIONS OF THIS DOCUMENT:

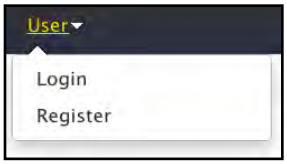
CloudMap | Cloud-based Pipeline for Analysis of Mutant Genome Sequences

UPLOADING FASTQ FILES (or any other type of file):

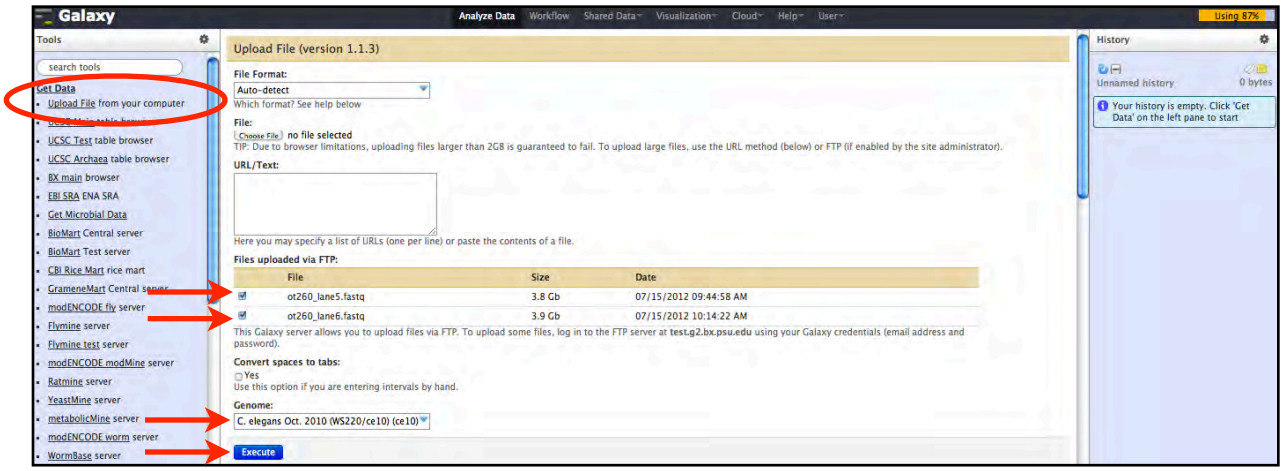
1) Navigate to the Galaxy site (http://usegalaxy.org)



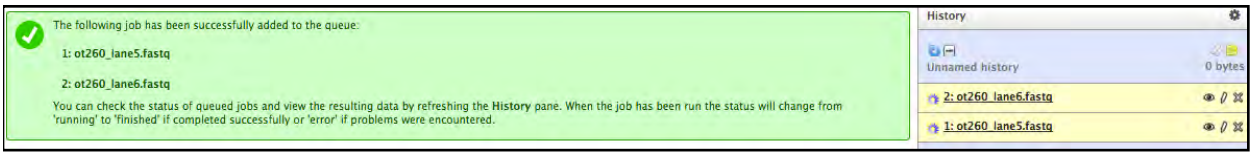
2) Register for an account or login if you already have an account:



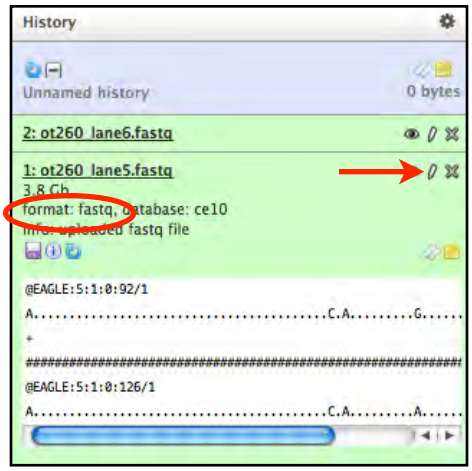
3) Once you are logged in using your email address, click on the ***Get Data*** link in the tools section on the left side of the screen. If the file you want to upload is < 2Gb, you can select the file through the ***Choose file*** link in the browser. Otherwise, you will need to upload your files via FTP (http://wiki.g2.bx.psu.edu/FTPUpload). If you upload your files via FTP, you will see the uploaded files in the ***Upload File*** browser window. Once the files have finished uploading via FTP, select them and the appropriate reference genome (***ce10*** for most of the examples in this user guide) and click ***Execute*** in order to add them to your history.



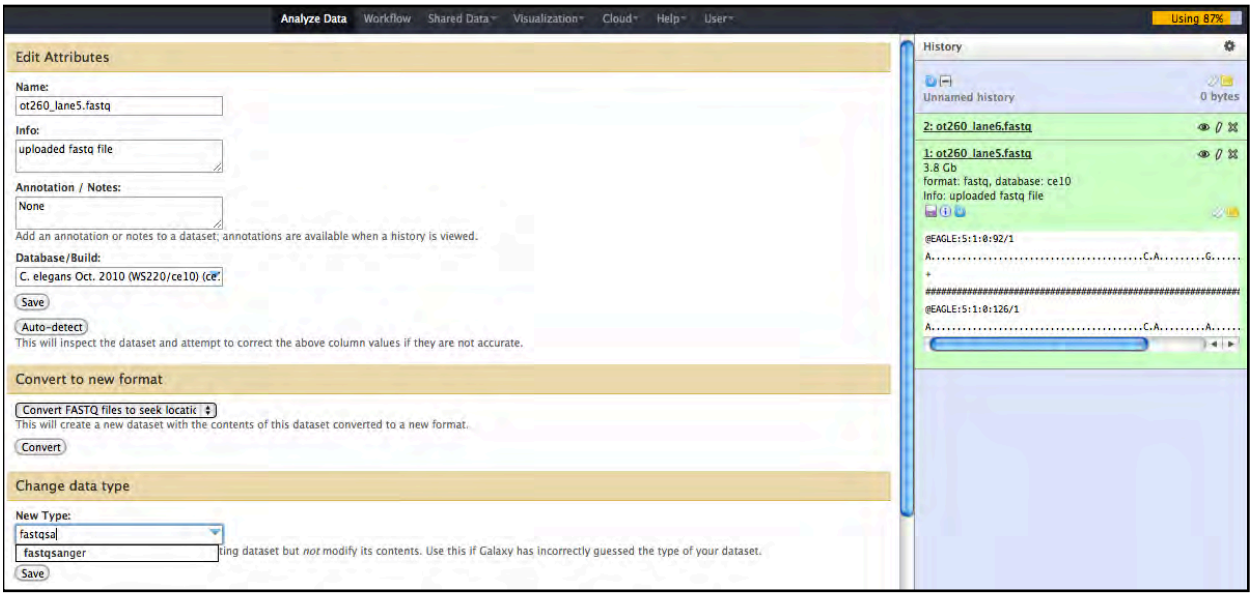
4) The files will be added to your history:



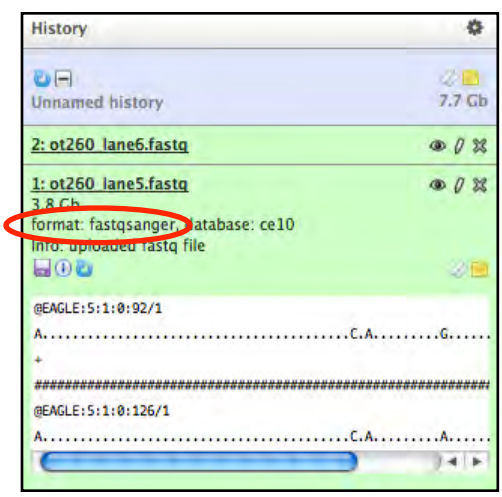
5) Once the FASTQ files are in your history, you will need to specify their data type (i.e. the base quality encoding scheme) by clicking on the file and then on the pencil icon:



6) The aligners in Galaxy accept the major FASTQ encoding schemes (fastqsanger and illumina) and FASTQ files can be converted from one format to another using the ***FASTQ Groomer*** tool. To read more about FASTQ encoding schemes, see the ***FASTQ Groomer*** tool or http://en.wikipedia.org/wiki/FASTQ_format

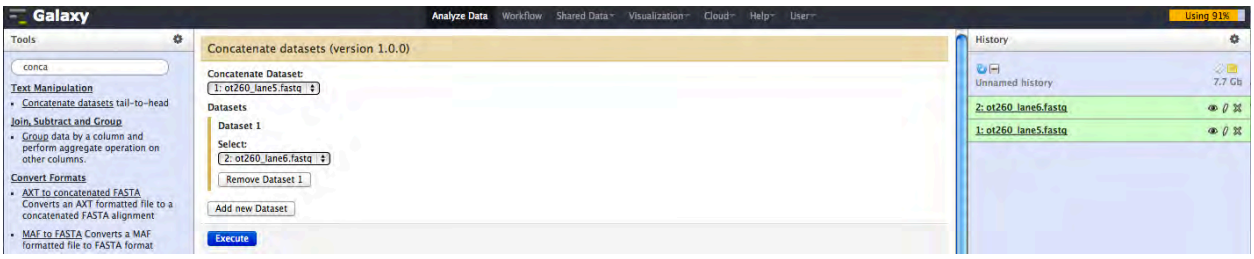


7) Your FASTQ file will now reflect the change. You can now proceed to import the various reference and configuration files required for the CloudMap workflows detailed elsewhere in this user guide.



CONCATENATING MULTIPLE FILES:

On occasion, your sample may be split up among multiple FASTQ files. In this case, you will need to concatenate your FASTQ files using the Galaxy ***Concatenate datasets*** tool:



You can now proceed to import the various reference and configuration files required for the CloudMap workflows detailed in this user guide.

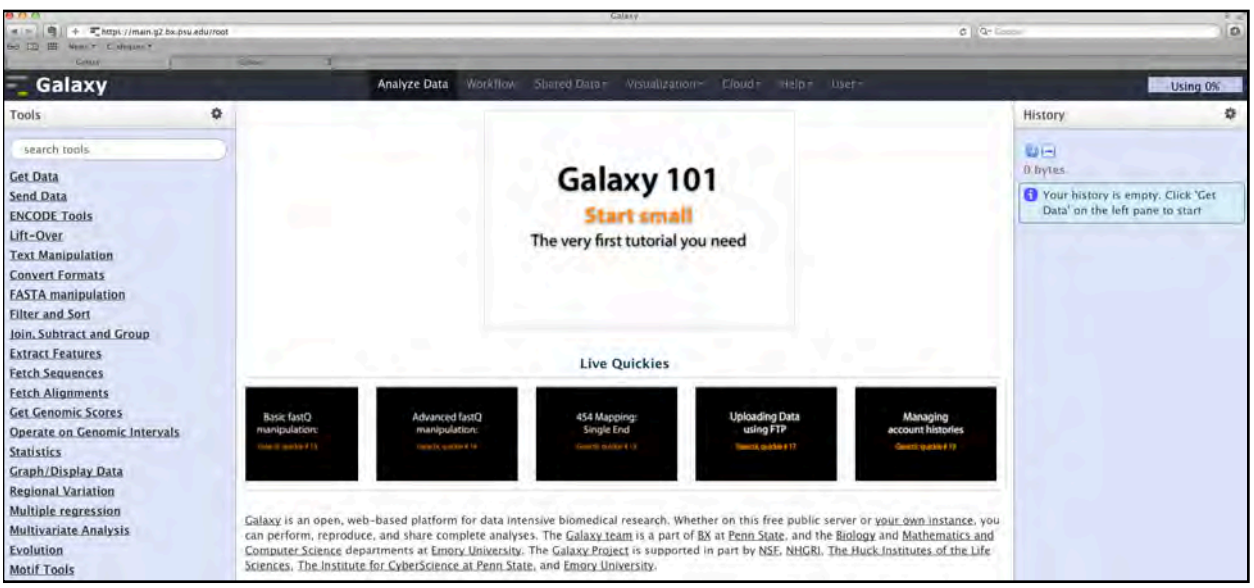
MODIFYING WORKFLOWS & CHANGING TOOL PARAMETERS (single-end vs paired-end data as an example):

The CloudMap workflows discussed in this user guide primarily describe how to run the ***ot266 Proof of principle***. However, these workflows can easily be edited to run any appropriate dataset. Here we will show you how to edit the ***CloudMap Hawaiian Variant Mapping with WGS Data and Variant Calling*** workflow to accept paired-end FASTQ data instead of single-end data. You can edit workflows to change parameters for each tool or to add new tools to your workflows.

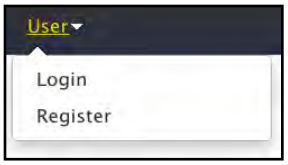
Useful workflow-related screencasts from Galaxy are available here:

- Create workflow from a history
- Create workflow from scratch
- Import workflow
- Edit workflow
- Convert workflow in a tool

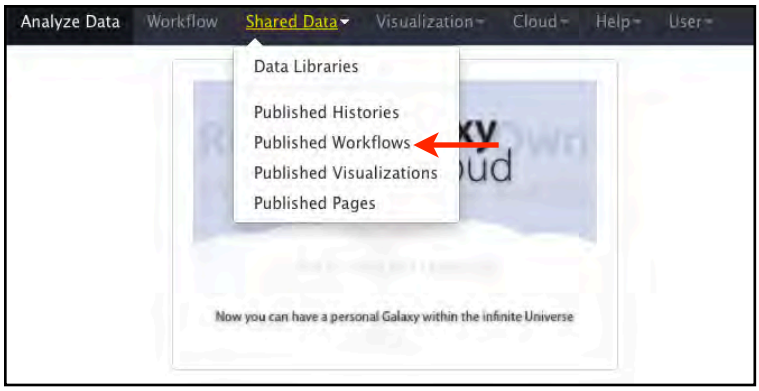
1) Let's assume that you haven't yet imported any CloudMap workflows. Navigate to http://usegalaxy.org/



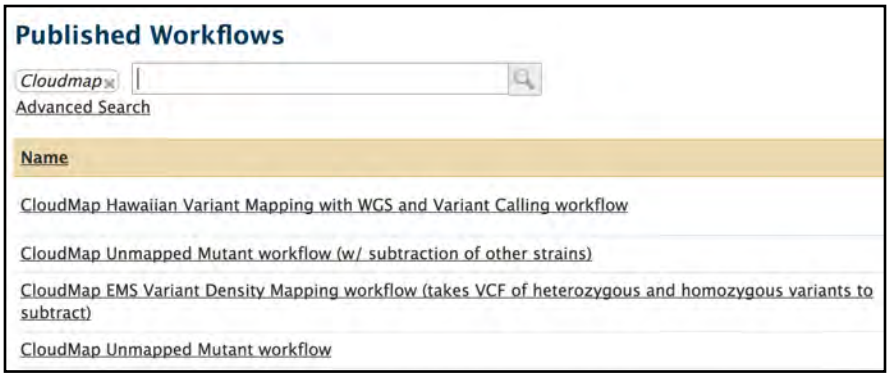
2) Register for an account or login if you already have an account:



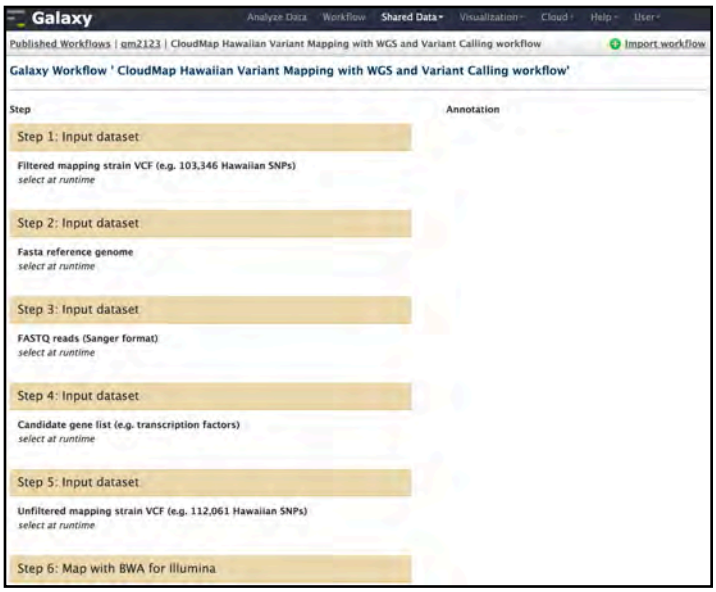
3) Click on the ***Shared Data*** link at the top of the page:



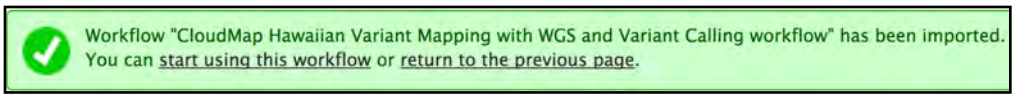
4) Click ***Published Workflows*** on the menu bar to access the automated workflow. Select the ***CloudMap Hawaiian Variant Mapping with WGS Data and Variant Calling workflow***.



5) You will now have the option to ***Import workflow***

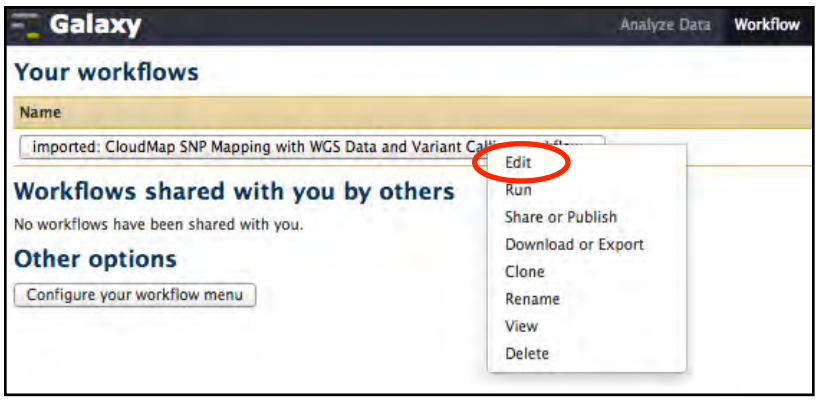


6) You will see this message:

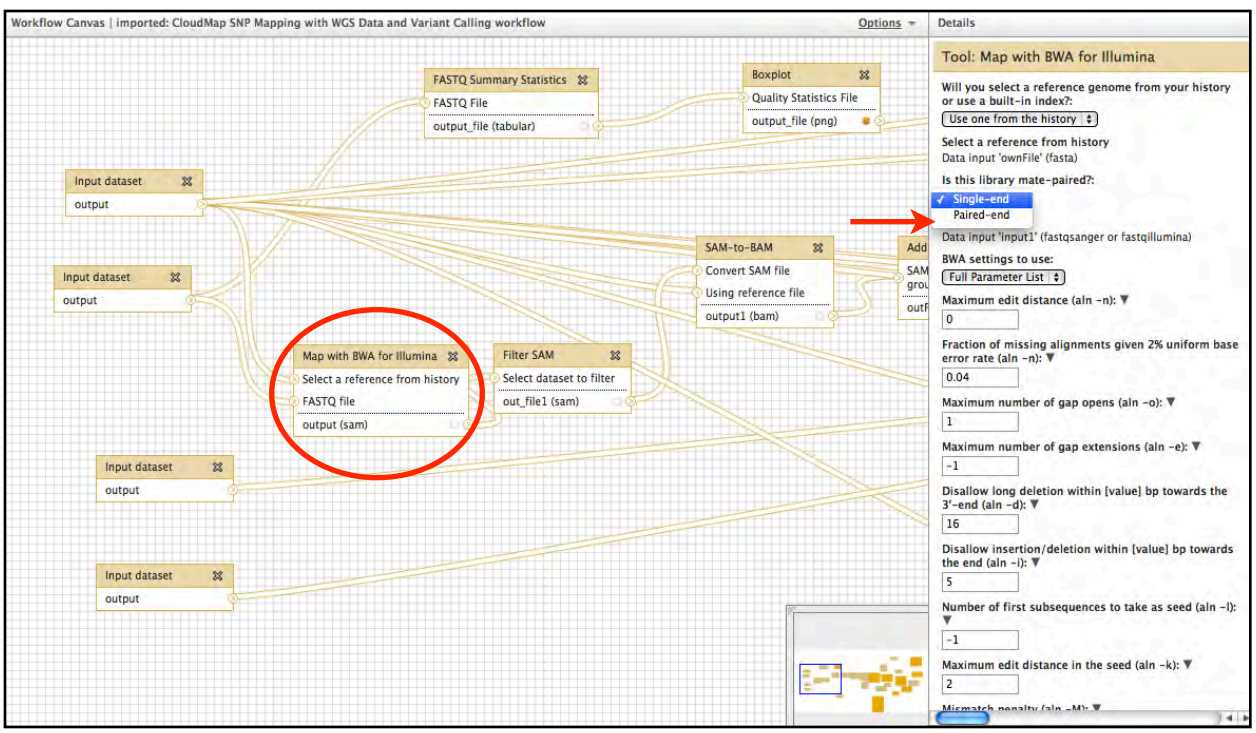


7) Click ***Start using this workflow*** and you will see that the workflow has been imported. From now on, you can easily access this workflow under the ***Workflow*** tab or in the Galaxy tools section (left frame of the browser window) under ***Workflows*** .

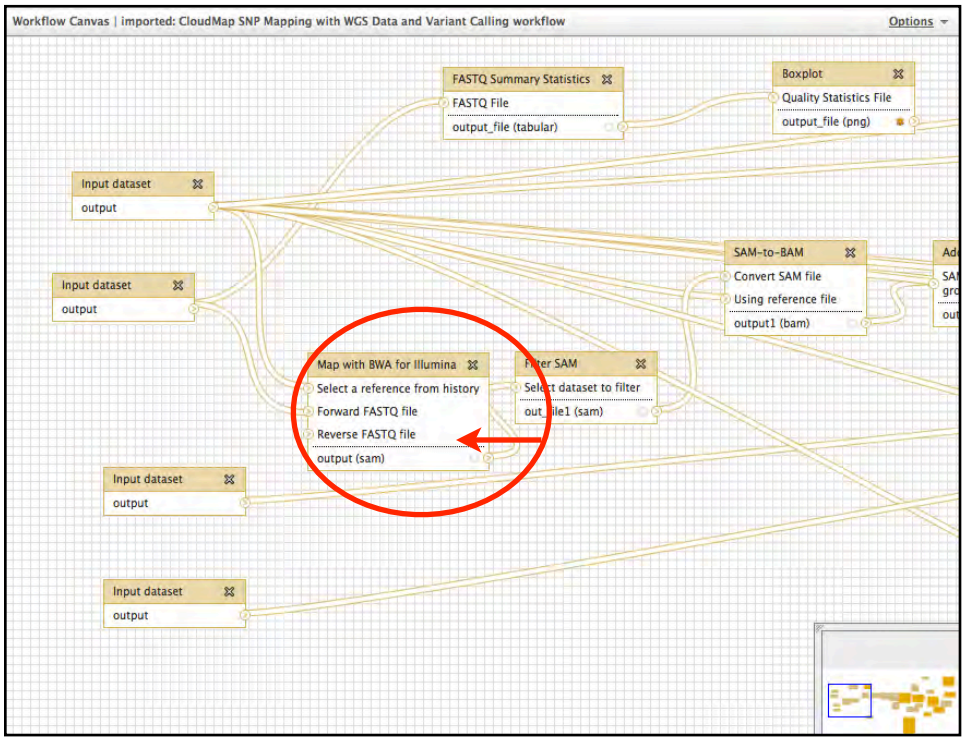
8) Click on the workflow and select ***Edit***:



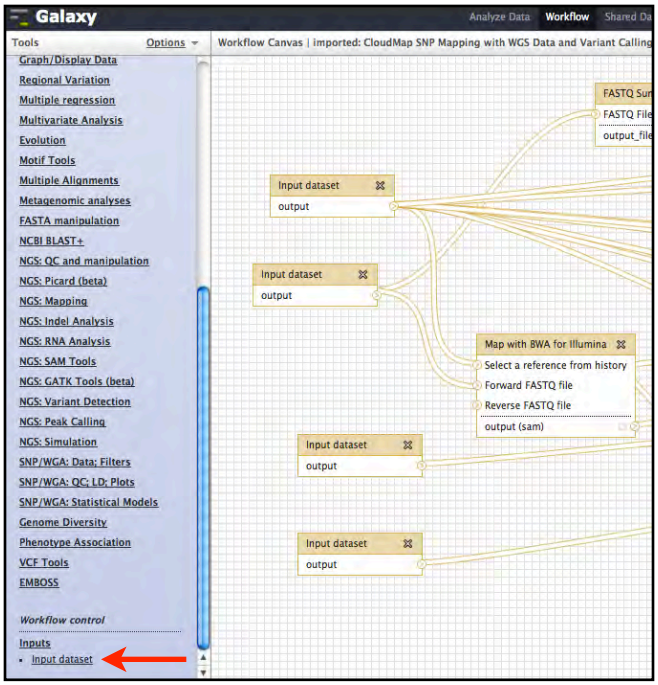
9) You will now see the workflow canvas that displays all the tools and input datasets in the workflow. By clicking on a given tool, you can change its parameters in the right frame of your browser window. We want to change the BWA mapping tool to accept paired-end data so we select the mapping tool and change the data input to paired-end:



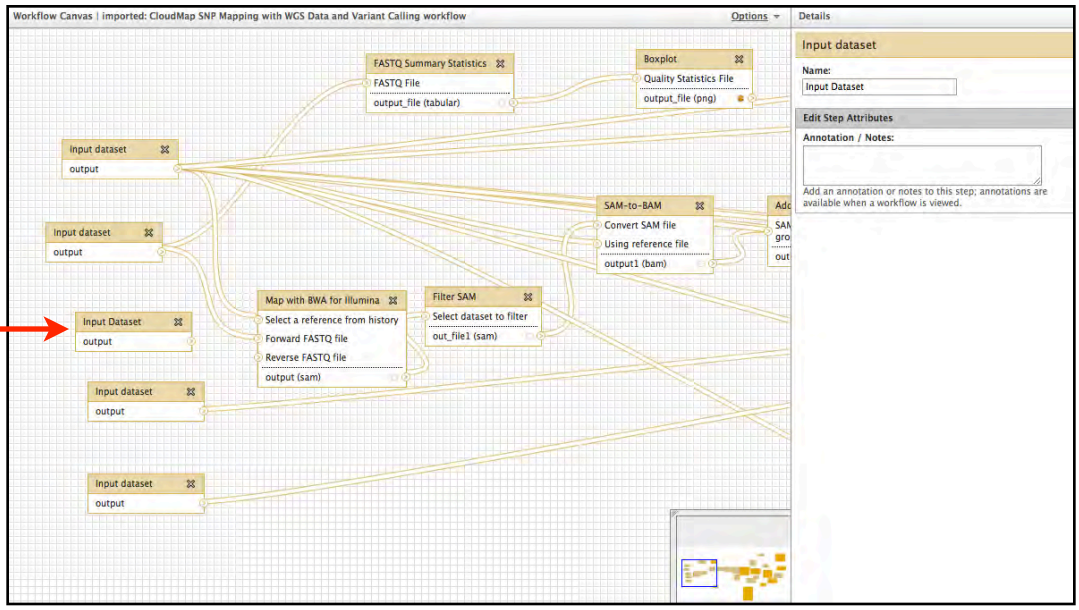
10) Once you select ***paired-end*** as the data type, the BWA mapping tool will now expect another input dataset.

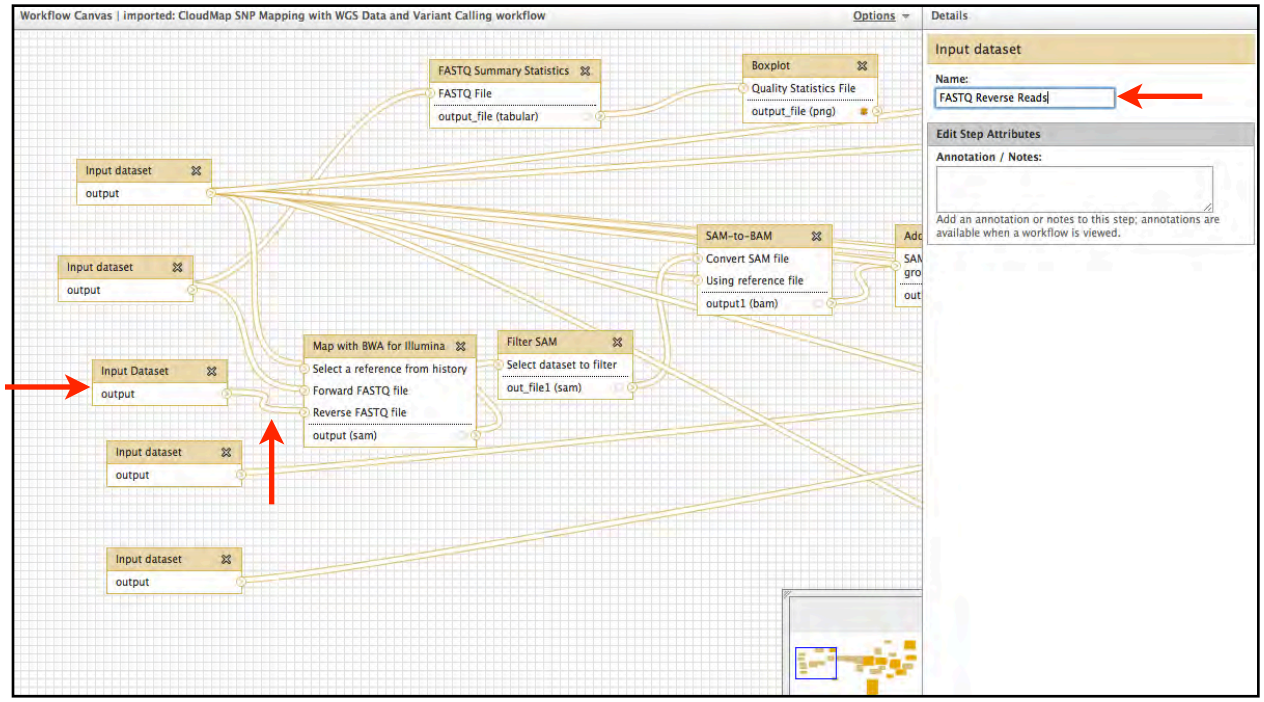


11) To add another input dataset, click ***input dataset*** under Galaxy tools:

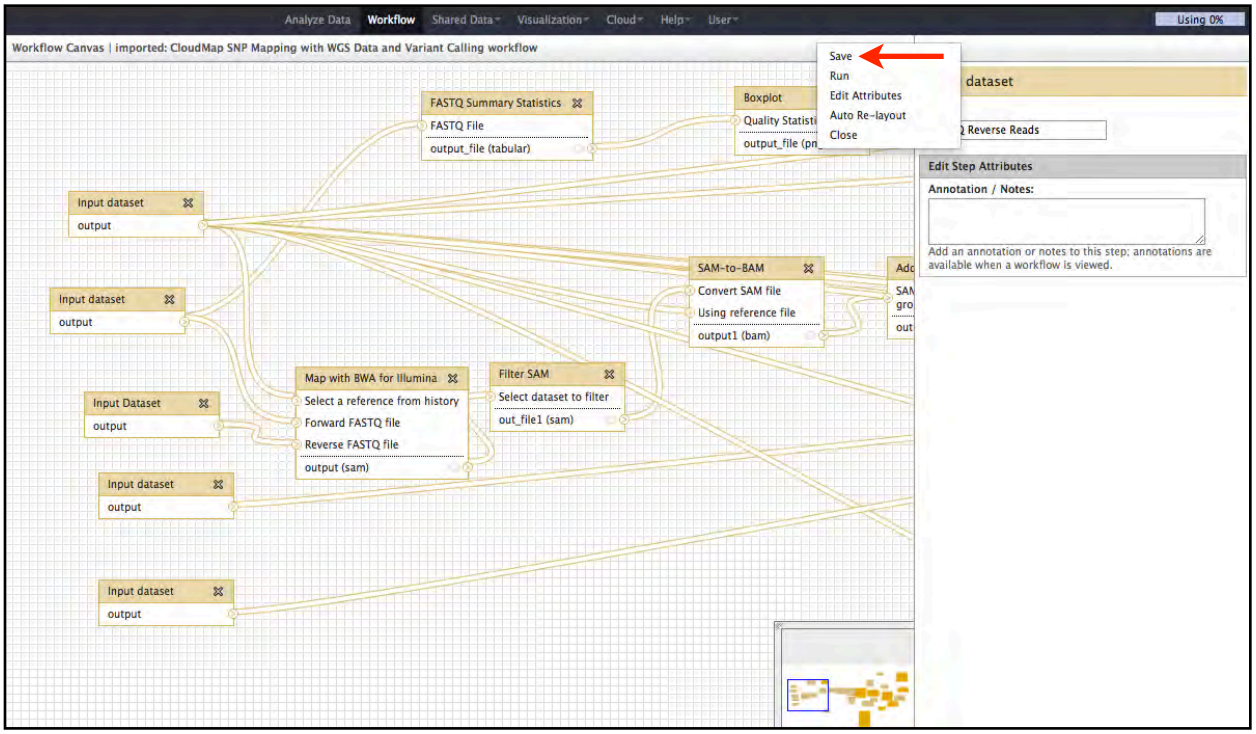


12) A new input dataset will appear in your workflow canvas. Attach the input dataset to the arrow next to ***Reverse FASTQ file*** in the ***Map with BWA for Illumina*** tool. If you don't have Illumina data, you can swap out the ***MAP with BWA for Illumina*** tool with one of the other aligners available within Galaxy. Make sure you give a name to your input dataset so you will know what data from your history should be matched to the input when you run the workflow:

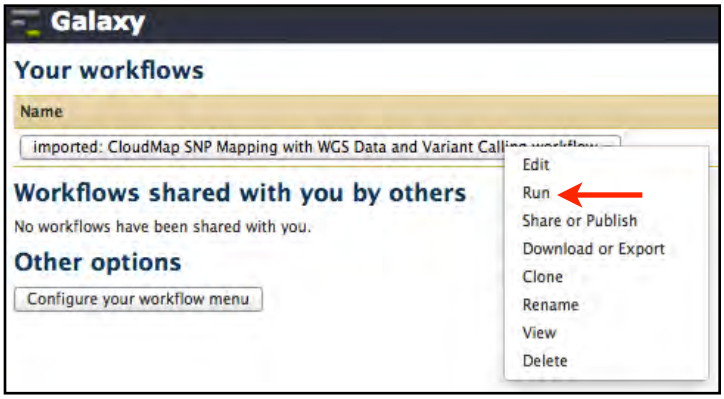




13) Now ***save*** the workflow and ***close***.



14) You can now run the modified workflow:



CONFIGURING THE *HAWAIIAN VARIANT MAPPING WITH WGS DATA WORKFLOW* TO SUPPORT SPECIES OTHER THAN *C.ELEGANS* AND *ARABIDOPSIS*:

1) Upload the Fasta reference file for the species you wish to analyze and a configuration file for the ***Hawaiian Variant Mapping with WGS Data*** tool. Refer to the ***UPLOADING FASTQ FILES (or any other type of file)*** section of this user guide for details on how to upload your own data. The configuration file is simply a two column, tab delimited list composed of the chromosome number and length in megabases. The numbering scheme of the chromosome should match that of the FASTA reference used for the analysis. Make sure that the FASTA headers (lines starting with >) contain only the chromosome name in one of the following formats:

>CHROMOSOME_<number>

>CHROM_<number>

><number>

i.e.:
>CHROMOSOME_1
>CHROM_1
>1

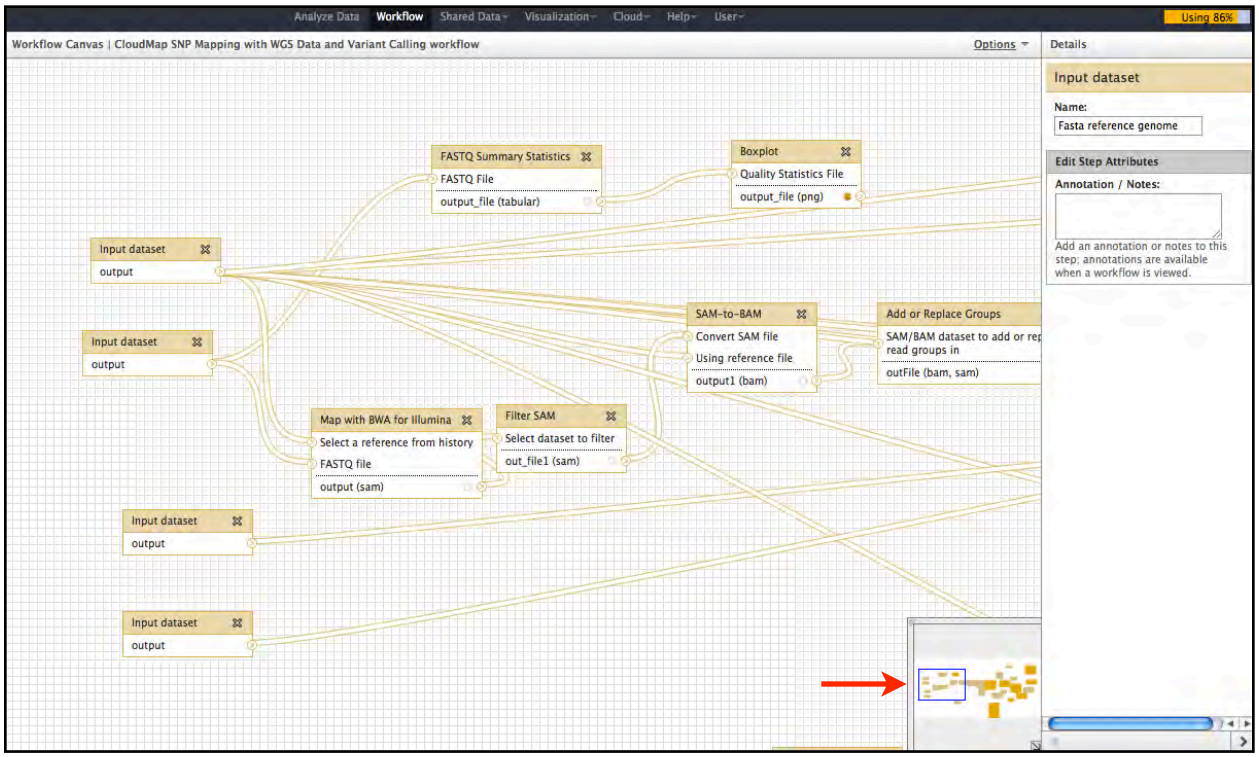
Sample *D.rerio* configuration file:

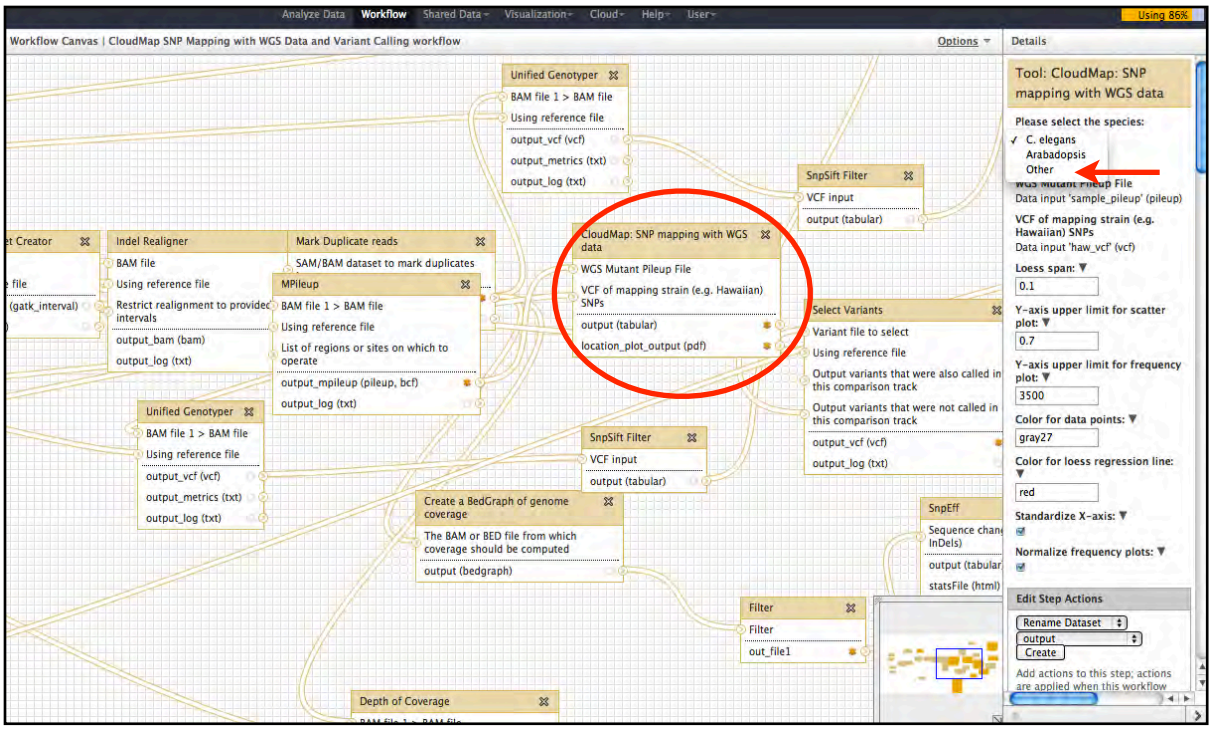
1	61
2	61
3	64
4	63
5	76
6	60
7	78
8	57
9	59
10	47
11	47
12	51
13	55
14	54
15	48
16	59
17	54
18	50
19	51
20	56
21	45
22	43
23	47
24	44
25	39

Please see more sample ***Other species*** configuration files in the CloudMap data library in the ***Hawaiian Variant Mapping with WGS Data Other Species Config Files*** folder.

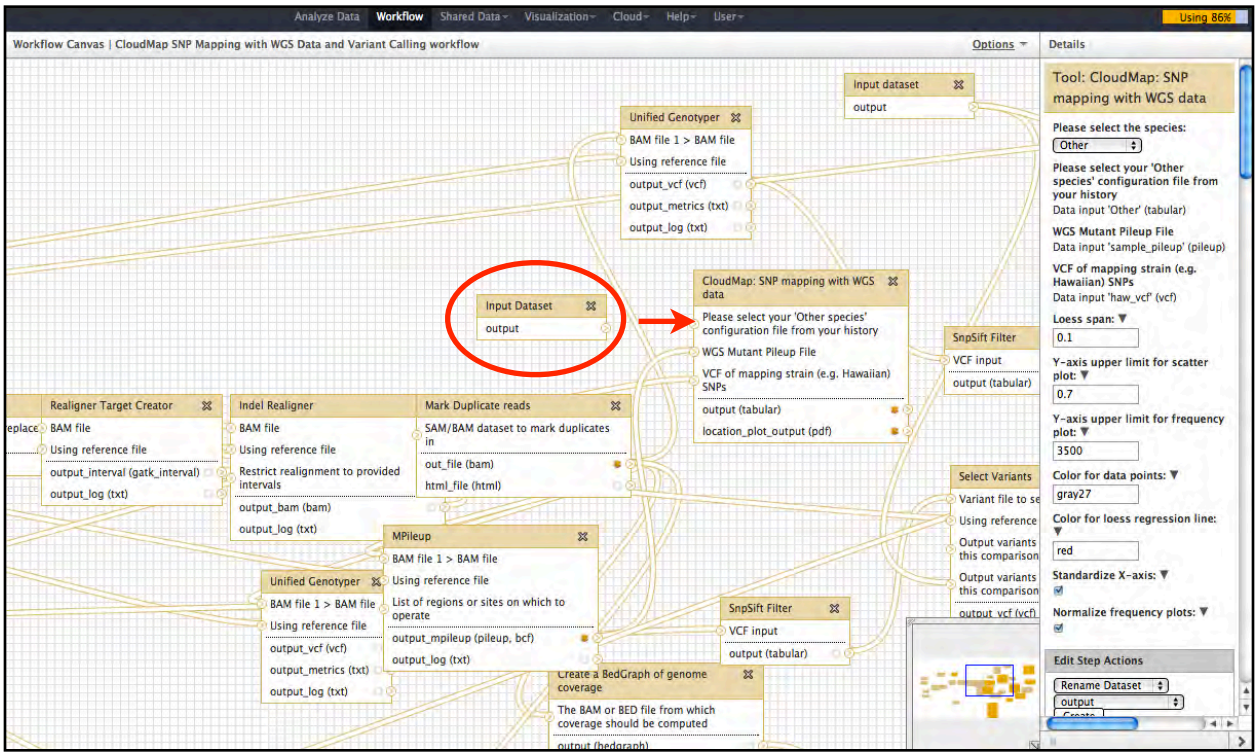
2) Now refer to steps 1-8 of the ***MODIFYING WORKFLOWS & CHANGING TOOL PARAMETERS*** section of this user guide to see how to edit the ***Hawaiian Variant Mapping with WGS Data and Variant Calling*** workflow. Step 3 below continues after step 8 of that workflow.

3) You should now see the workflow canvas that displays all the tools and input datasets in the workflow. Scroll across the window displaying all of the tools in the workflow by dragging the small square at the bottom right of your window.

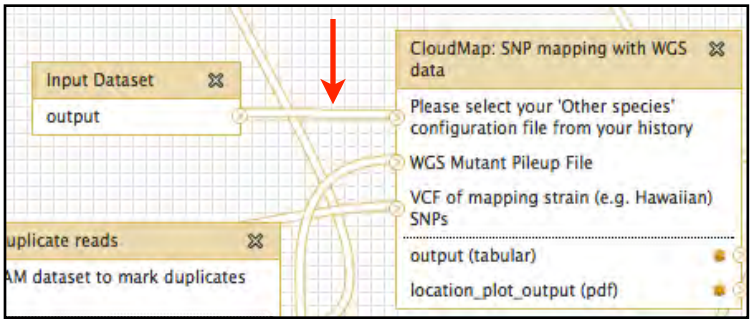




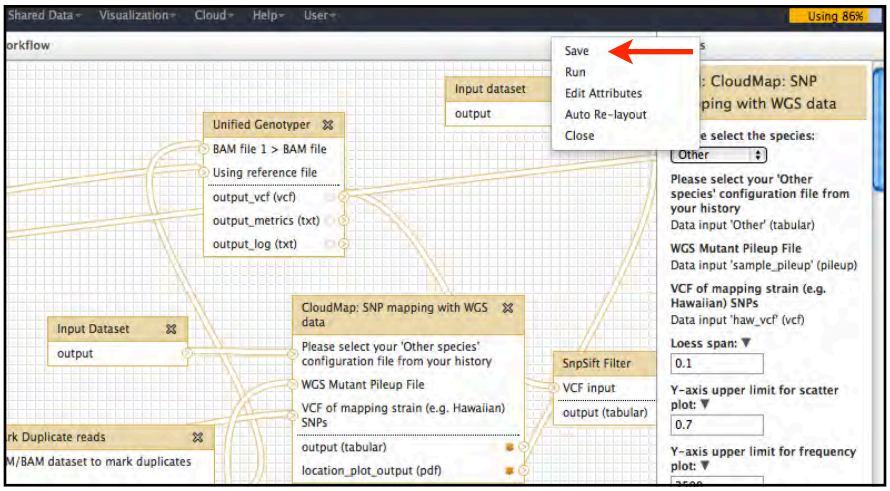
4) Select the ***CloudMap Hawaiian Variant Mapping with WGS Data*** tool, then select ***Other*** from species list.



6) Connect the ***Other species*** input dataset to the ***CloudMap Hawaiian Variant Mapping with WGS Data*** tool by clicking and dragging the arrow on the side of the Input dataset tool.



7) Now save and close the workflow and you're ready to run it.



CloudMap | Cloud-based Pipeline for Analysis of Mutant Genome Sequences

This document contains **Frequently Asked Questions** (FAQs) regarding CloudMap and Galaxy. The document will be continually updated. For more details, please see the CloudMap paper or visit the CloudMap website at: http://usegalaxy.org/cloudmap. Video versions of these user guides are available at the CloudMap website.

Your first stop for Galaxy-related FAQs:
http://wiki.g2.bx.psu.edu/Support
http://wiki.g2.bx.psu.edu/Learn/FAQ

http://seqanswers.com/ is a very useful next generation sequencing forum.

FAQs:

Cloudmap questions:

1) My workflow is missing steps mentioned in the user guide, how do I get the latest version?

2) I would like to change some aspect of the plots, how can I do this?

Galaxy questions:

1) My tool turned red after execution and no output file was created. What should I do?

2) I see my data in my history but the tool won't recognize it. What's wrong?

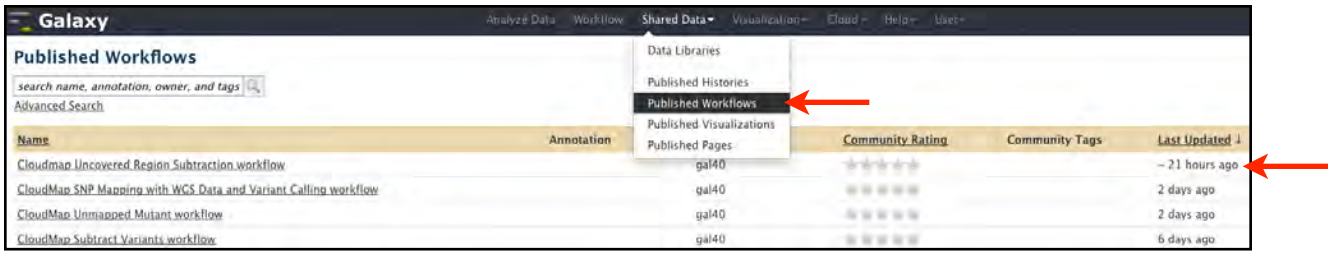
3) I want to use a specific genome build that isn't available in Galaxy. How can I do this?

Cloudmap questions:

My workflow is missing steps mentioned in the user guide, how do I get the latest version?

Make sure you re-import your workflows to get the latest versions. Check under

Shared Data —> Published Workflows to see when workflow were last updated.



I would like to change some aspect of the plots, how can I do this?

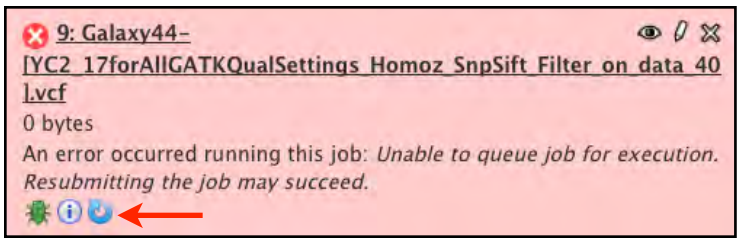
You can email us with your request at gm2123@columbia.edu or or38@columbia.edu. If you want to make the change yourself and run the tool locally, you can download the source code from the Galaxy Tool Shed at: http://toolshed.g2.bx.psu.edu/

Read more about the Galaxy Tool Shed here: http://wiki.g2.bx.psu.edu/Tool%20Shed

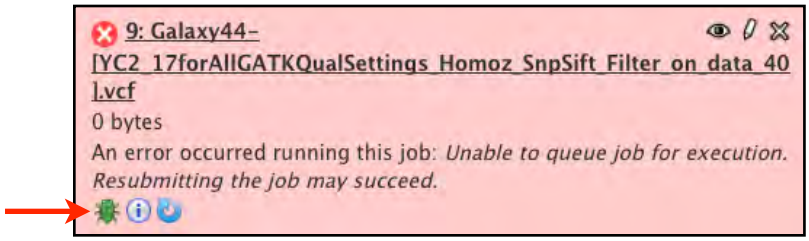
Galaxy questions:

My tool turned red after execution and no output file was created. What should I do?

First check that you provided the correct type of input file and settings for the tool. Next try rerunning the tool by clicking the ***run this job again*** arrow.



Failing that, submit a bug report to Galaxy by clicking on the bug icon.



I see my data in my history but the tool won't recognize it. What's wrong?

This is one of the most common problems users encounter within Galaxy. Use the pencil icon to change the data type to the correct type. http://wiki.g2.bx.psu.edu/Learn/Managing%20Datasets

I want to use a specific genome build that isn't available in Galaxy. How can I do this?

For the vast majority of the tools (BWA, Bowtie aligners especially), you can upload genome reference files (FASTA) and use these for the duration of the history. If you're using a tool that only takes genome builds that are "hard-coded" within Galaxy and you want to support a specific genome, please check the Galaxy support page: http://wiki.g2.bx.psu.edu/Support.

If you plan to use an uploaded FASTA file with the ***Hawaiian Variant Mapping with WGS Data*** tool, make sure that the FASTA headers (lines starting with >) contain only the chromosome name in one of the following formats:

>CHROMOSOME_<number>

>CHROM_<number>

><number>

If you plan to use an uploaded FASTA file with the ***Hawaiian Variant Mapping with WGS Data*** tool and your FASTA file is for a species other than *C.elegans* or *Arabidopsis*, make sure the chromosome naming convention in the ***Other species*** configuration file matches that of the FASTA file. Please see sample ***Other species*** configuration files in the CloudMap data library in the ***Hawaiian Variant Mapping with WGS Data Other Species Config Files*** folder.