	Α	В
1	C35E7.2	2
2	Y8A9A.2	2
3	Y16B4A.2	2

В

Α

	A	B		D	E	F	G	н	1	J	K	L	M	N	0	P	Q	R	S	T	U	V
1	Sample	# Chromo	Position	Reference	Change	Change_type	Homozygous	Quality	Coverage	Warnings	Gene_ID	Gene_name	Bio_type	Trancript_IC	D Exon_ID	Exon_Rank	Effect	old_AA/new_A	A Old_codon/New_codon	Codon_Num	Codon_Dege (DS_size
2	mutA	1	10841384	G	С	SNP	Hom	43.12	2		C35E7.2	C35E7.2	protein_coding	C35E7.2a	exon_l_10841103_10841965	1	NON_SYNONYMOUS_CODING	R/T	aGa/aCa	92	0	2124
3	mutB	1	10841434	Α	С	SNP	Hom	80.72	3		C35E7.2	C35E7.2	protein_coding	C35E7.2a	exon_l_10841103_10841965	1	NON_SYNONYMOUS_CODING	I/L	Att/Ctt	109	0	2124
4	mutB	11	3796684	C	Α	SNP	Hom	349.22	21		Y8A9A.2	Y8A9A.2	protein_coding	Y8A9A.2	exon_II_3796348_3797638	5	NON_SYNONYMOUS_CODING	P/Q	cCa/cAa	286	0	4083
5	mutC	11	3796759	Α	т	SNP	Hom	1208.35	56		Y8A9A.2	Y8A9A.2	protein_coding	Y8A9A.2	exon_II_3796348_3797638	5	NON_SYNONYMOUS_CODING	N/I	aAt/aTt	311	0	4083
6	mutA	X	14766637	G	Α	SNP	Hom	44.89	4		Y16B4A.2	Y16B4A.2	protein_coding	Y16B4A.2	exon_X_14766327_14766971	18	NON_SYNONYMOUS_CODING	S/F	tCc/tTc	1073	0	6504
7	mutB	X	14766625	•	—G	DEL	Hom	506.78	21		Y16B4A.2	Y16B4A.2	protein_coding	Y16B4A.2	exon_X_14766327_14766971	18	FRAME_SHIFT: Y16B4A.2					6504

Figure S1 CloudMap *in silico Complementation Test* tool. **A:** Summary output. CloudMap allows for large scale *in silico* comparison of annotated WGS variants (that have been filtered for quality and had common variants subtracted) between many samples. The summary output from this comparison shows the number of alleles of each gene sorted from most to fewest. **B:** Comprehensive output. For each in silico Complementation Test summary output file, CloudMap provides the corresponding detailed list of snpEff-annotated, allelic gene hits that is also sorted from most to fewest alleles.



Figure S2 Uncovered region confirmed to be a genomic deletion. CloudMap contains a workflow for annotating uncovered regions that may be genomic deletions. Users are encouraged to check if uncovered regions repeatedly appear in other strains and also to view these putative deletions in an alignment viewer. We find that true deletions tend to exhibit a cliff of high coverage followed by zero coverage on both uncovered boundary regions. Regions of high coverage flanking the putative deletion also often have SNPs or insertions present in many of the reads — indicating that distant genomic regions are now adjacent to one another. The deletion shown was confirmed to be a deletion via PCR and Sanger sequencing. The IGV viewer is used to display the alignment (Robinson et al., 2011, *Nature Biotechnology* 29, 24–26).



Figure S3 *EMS Variant Density Mapping* tool: CloudMap also supports the approach detailed in Zuryn et al., that involves plotting frequencies of variant density in a mutant *C. elegans* strain that has been backcrossed to its (pre-mutagenesis) starting strain (ZURYN *et al.* 2010).



Figure S4 *Variant Discovery Mapping* normalization equation. Pure parental alleles are defined as those positions in the pooled sequenced mutant where variant reads/total reads = 1 (after the appropriate variant subtraction strategy has been applied). Normalization is applied by default although users have the option of turning it off.



Figure S5 Multi-organism *Variant Discovery Mapping* support. CloudMap natively supports the variant discovery mapping method for *Arabidopsis* as shown here for data from Schneeberger *et al.* 2009. Users must provide a simple configuration file for organisms other than C. elegans and Arabidopsis. Configuration files and instructions for other organism support are provided at http://usegalaxy.org/cloudmap

 Table S1
 WS220 Hawaiian Variants filtered to assist in CloudMap Hawaiian Variant Mapping with WGS Data plot normalization. This table provides details on the numbers of Hawaiian SNPs subtracted from mapping analysis for purposes of SNP mapping plot normalization. Details provided in text and in Fig.7.

Mutant	Trans-gene	Location of trans- gene (LG)	Location of Mutation (LG)	LG I	LG II	LG III	LG IV	LG V	LG X	< .05 or > .95 ratio positions	< .05 or > .95 ratio positions after transgene & mutation LGs removed
ot219	otls114	I	V		1	1	1		1	15,873	4,003
ot266	vt/s1	V	х	1	1	1	1		✓*	13,669	11,033
	oxls12,				1	1	1				
ot628	vsls33	V, X	I, V							22,268	3,367
	otls138,			1	1	1	1				
ot641	vls33	V <i>,</i> X	Х							9,616	4,314
	otls138,			1	1	1	\checkmark				
ot642	vs133	V, X	Х						√ **	13,589	5,021
ot704	otls341	Х	L		1	1	1	1		14,805	7,197
ot705	otls341	х	111	1	1		1	1		14,686	7,969

✓ Considered SNPs on this chromosome for filtering

* Excluding 6-13 Mb

** Excluding 0-6 Mb

Total dataset used:

WS220_WormMart HA SNPs: 112,061 Variants

<.05 or >.95 ratio positions in at least 2 samples: 8,715 Variants

<.05 & >.95 ratio positions in all 7 samples:1,563 Variants

Final curated Hawaiian SNP list (WS220.64): 103,346 Variants

	MAQGene	CloudMap	
# of pooled recombinants	50	50	
Defined mapping interval	8,841,415-10,975,250	10,000,000-11,000,000	
	(aligned to WS201)	(aligned to WS220)	
Defined mapping region in Mb	2.13	1	
# Variants in the region	26	22	
(pre-variant subtraction)			
# Variants in the region	not performed	10	
(post- variant subtraction)			
# of protein coding variants in respective mapping regions	3	2	
Premature stops	1	1	

Table S2 CloudMap comparison with MAQGene. This table shows variants in the mapping region of *ot266* as determined by CloudMap vs. MAQGene. CloudMap was able to identify a smaller mapping region than MAQGene (1Mb vs. 2.13Mb).

Additional Supporting Materials

Video user guides, automated workflows, and up to date CloudMap tools are available at: http://usegalaxy.org/cloudmap