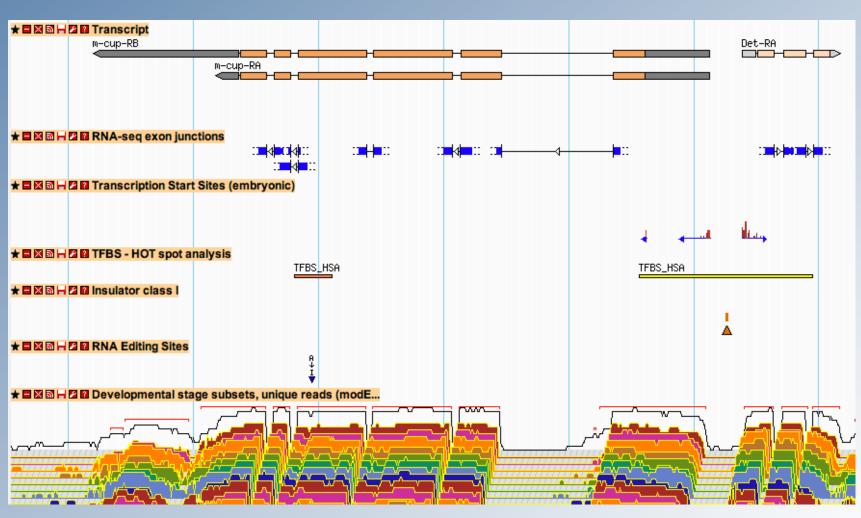
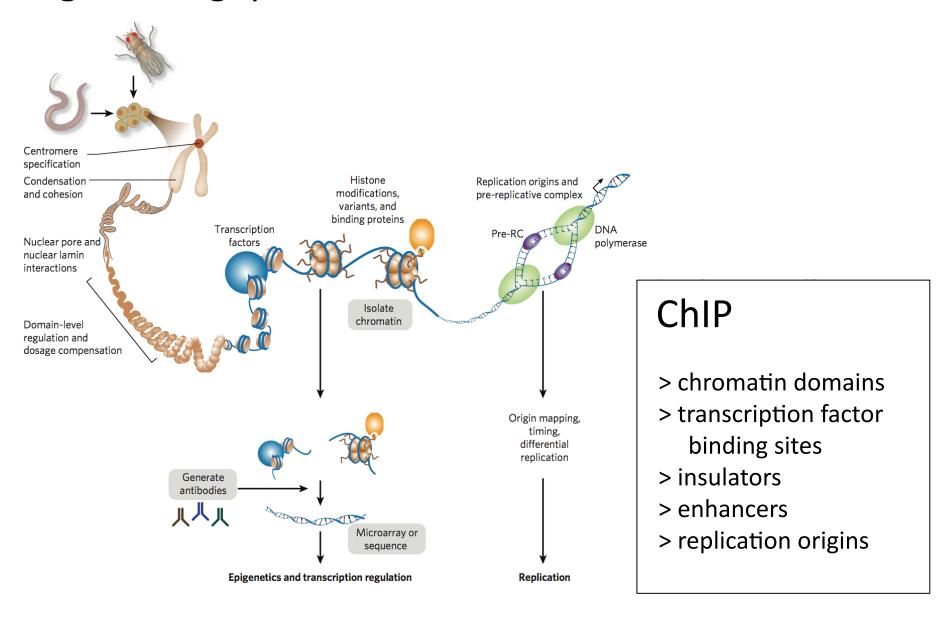


Navigating High-throughput Data in FlyBase



High-throughput data overview

(Celniker, 2009)

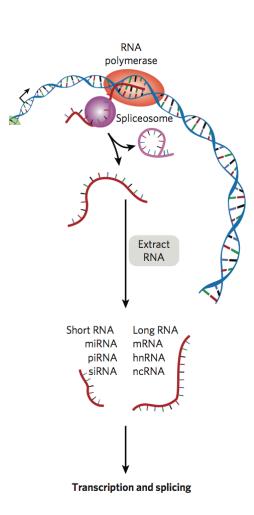


High-throughput data overview

(Celniker, 2009)

RNA-Seq

- > transcriptome
- > exon junctions
- > A-I editing sites
- > transcription starts
- > new genes



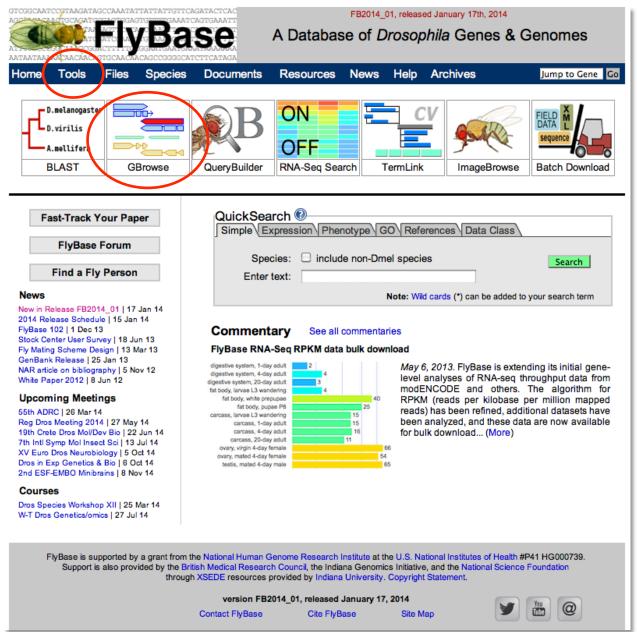
High-throughput Data: Views, reports, and queries

- GBrowse options (GBrowse 2)
- Dataset reports (large dataset metadata)
 - Links to GEO and modMine
- Sequence feature reports
- FeatureMapper
- Querying RNA-Seq expression data

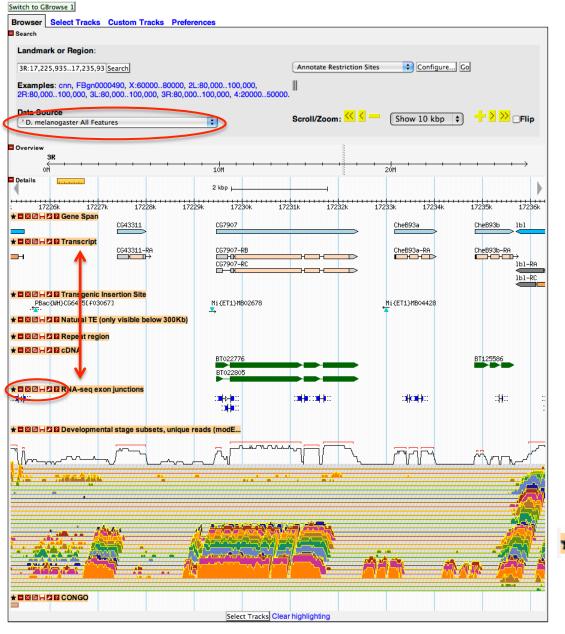
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High-throughput data in GBrowse: getting there



Navigating GBrowse 2: Important new features



- Data Source menu new 'All Features' view
- Easy to delete or hide track data
- Tracks are moveable up and down
- Links to information/ metadata for tracks (here and on Tracks page)
- Select Tracks two links.

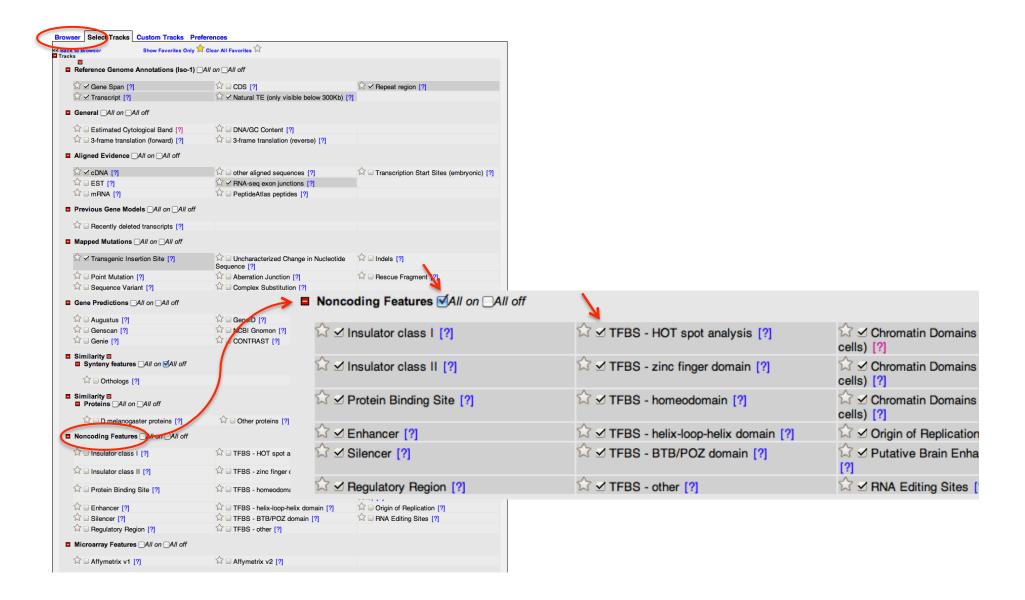


Navigating GBrowse 2: Important new features



- Data Source menu new 'All Features' view
- Easy to delete or hide track data
- Tracks are moveable up and down
- Links to information/ metadata for tracks (here and on Tracks page)
- Select Tracks two links.

Navigating GBrowse 2: Track Selection



GBrowse tracks documentation

■ Noncoding Features □All on □All off		
☆ ☐ Insulator class I [?]	TFBS - HOT spot analysis [?]	☐ Chromatin Domains (5-state model, Kc cells) [?]
☆ ☐ Insulator class II [?]	TFBS - zinc finger domain [?]	☐ Chromatin Domains (9-state model, S2 cells) [?]
☆ ☐ Protein Binding Site [?]	TFBS - homeodomain [?]	☐ Chromatin Domains (9-state model, BG3 cells) [?]
☆ □ Enhancer [?]	TFBS - helix-loop-helix domain [?]	☐ Origin of Replication [?]
☆ Silencer [?]	☆ □ TFBS - BTB/POZ domain [?]	RNA Editing Sites [?]
Regulatory Region [?]	☆ ☐ TFBS - other [?]	
■ Microarray Features □All on □All off		
Affymetrix v1 [?]	☐ Affymetrix v2 [?]	
■ Expression Levels ■ MicroArrays All on All off		
Timecourse [cDNA] (Arbeitman et al.)	Timecourse [amplicons] (Gauhar et al.)	☆ ☐ Tissue Expression [Affy2] (FlyAtlas) [?]
■ Expression Levels ☑■ RNA-seq □All on □All off		
☐ Developmental stage subsets (Baylor)[?]	Transcription Group) [?]	Treatments/Conditions, by strand [?]
	Tissue culture cells, by strand [?] (modENCODE Transcription Group)	
■ Expression Levels ■ RNA-Seq by Tissue All on All off		
☆ □ Digestive system [?]	CNS and adult head [?]	☆ □ L3 CNS neuroblast [?]
☐ Fat body and salivary glands [?]	☐ Gonads and male accessory glands [?]	
☆ □ Imaginal disc and other carcass [?]	□ L3 CNS neuron [?]	

GBrowse tracks documentation: tracks = datasets

Expression Levels: RNA-Seq

Developmental stage subsets (Baylor) FBIc0000060

Available in these data source views:

Expression/Regulation

Developmental stage subsets, unique reads (modENCODE) FBIc0000085

Available in these data source views:

· Expression/Regulation

Tissue culture cells (modENCODE Transcription Group) FBIc0000116

Available in these data source views:

Expression/Regulation

Tissue culture cells, by strand (modENCODE Transcription Group) FBIc0000260

Available in these data source views:

Expression/Regulation

Treatments/Conditions FBIc0000236

Available in these data source views:

Expression/Regulation

Expression Levels: RNA-Seq by Tissue

Digestive system Comprised of four BNA-Seq data sets: mE_mpNA_L3_Wand_dig_sys (FBlc0000227 mE_mRNA_A_1d_dig_sys FBlc0000229 mE_mRNA_A_4d_dig_sys FBlc0000223 mE_mRNA_A_2dd_dig_sys FBlc0000221

Available in these data source views:

Expression/Regulation

Fat body and salivary glands mE_mRNA_L3_Wand_fat FBlc0000228 mE_mRNA_WPP_fat FBlc0000233 mE_mRNA_P8_fat FBlc0000235 mE_mRNA_L3_Wand_saliv FBlc0000230 mE_mRNA_WPP_saliv FBlc0000234

Available in these data source views:

Expression/Regulation

Imaginal disc and other carcass mE_mRNA_L3_Wand_imag_disc FBIc0000229 mE_mRNA_L3_Wand_carcass FBIc0000226 mE_mRNA_A_1d_carcass FBIc0000218 mE_mRNA_A_4d_carcass FBIc0000222 mE_mRNA_A_20d_carcass FBIc0000220

Available in these data source views:

Expression/Regulation

CNS and adult head mE_mRNA_L3_CNS FBIc0000225 mE_mRNA_P8_CNS FBIc0000224 mE_mRNA_A_MateM_1d_head FBIc0000209 mE_mRNA_A_MateM_4d_head FBIc0000216 mE_mRNA_A_MateM_20d_head FBIc0000214 mE_mRNA_A_VirF_1d_head FBIc0000210 mE_mRNA_A_VirF_4d_head FBIc0000211 mE_mRNA_A_VirF_20d_head FBIc0000231 mE_mRNA_A_MateF_1d_head FBIc0000207 mE_mRNA_A_MateF_4d_head FBIc0000213 mE_mRNA_A_MateF_20d_head FBIc0000212

Available in these data source views:

· Expression/Regulation

Gonads and male accessory glands mE_mRNA_A_MateM_4d_testis FBic0000217 mE_mRNA_A_MateM_4d_acc_gland FBic0000215 mE_mRNA_A_VirF_4d_ovary FBic0000232 mE_mRNA_A_MateF_4d_ovary FBic0000238

Available in these data source views:

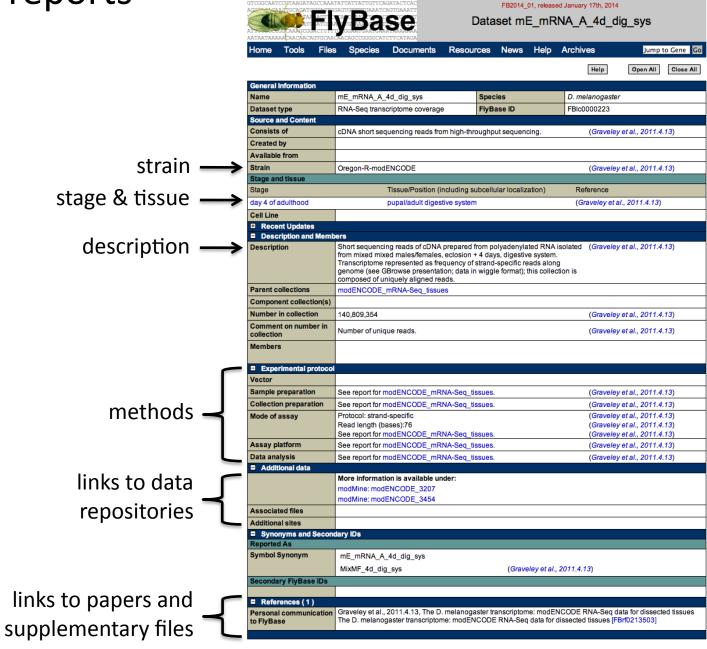
Expression/Regulation

To Dataset report

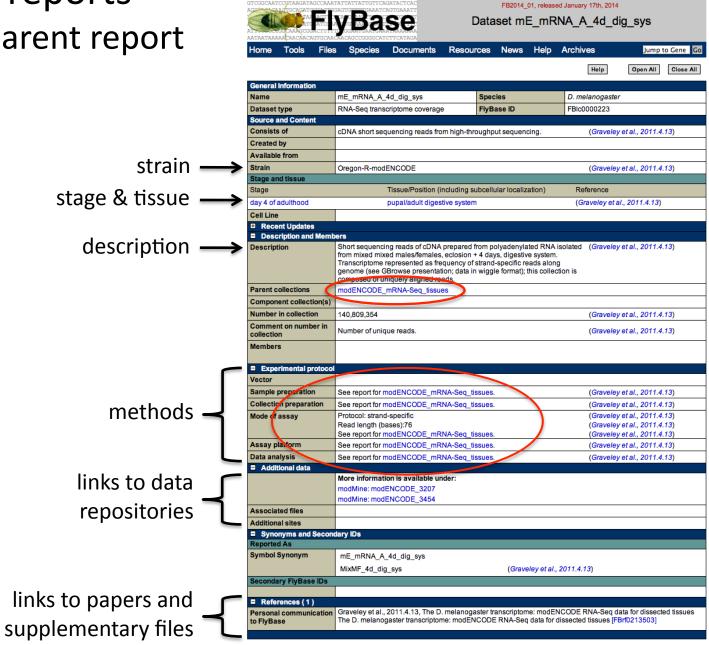
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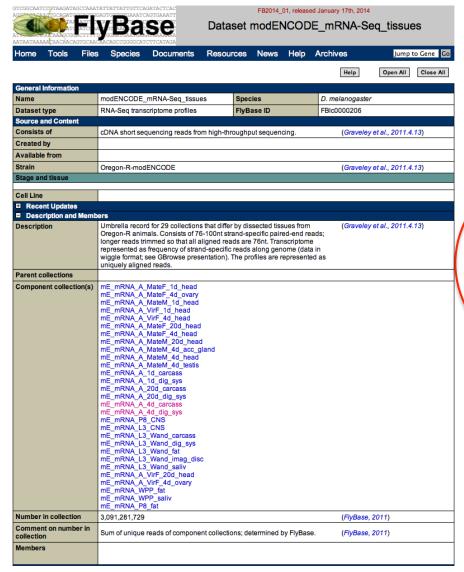
Dataset reports



Dataset reports – links to parent report

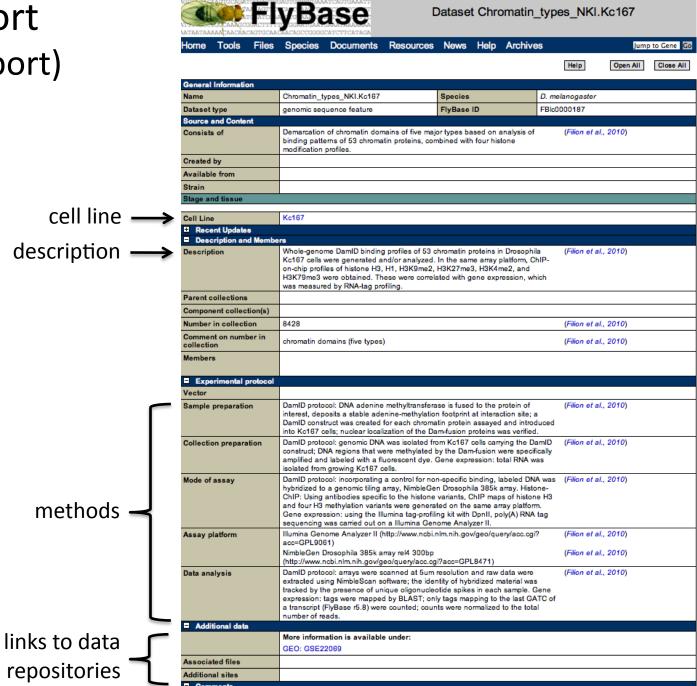


Dataset reports – Parent report



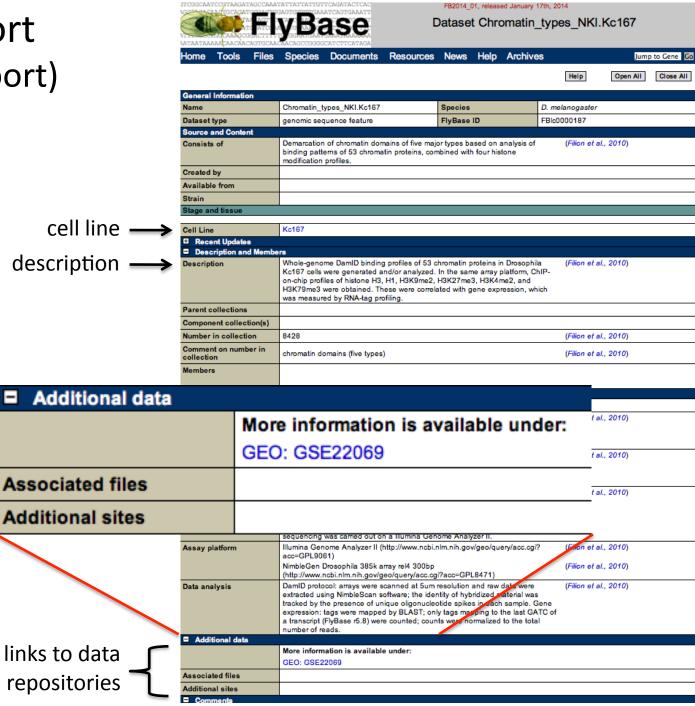
Vector		
Sample preparation	Collection of tissues and preparation of total RNA samples described in modMine entry modENCODE 3207 (link below).	Graveley et al., 2011.4.13)
Collection preparation	Polyadenylated RNAs were purified from total RNA extracts via oligo(dT) binding, using standard Illumina protocol. The poly(A)+ RNA was fragmented using divalent cations under elevated temperature, following by sequential ligation of RNA linkers to the 5' and 3' ends. Next, reverse transcription was performed using a primer complementary to the 3' linker and PCR was performed using primers complementary to both linkers. ~ 300 bp fragments were isolated from an agarose gel and gel-purified again.	(Grave) ey et al., 2011.4.13)
Mode of assay	The samples were quantitated using a Nanodrop, and loaded onto a flow cell for cluster generation and sequenced on an Illumina Genome Analyzer II or HISeq 2000 using paired end protocols (Illumina).	(Graveley et al., 2011.4.13)
Assay platform	Illumina Genome Analyzer II (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi? acc=GPL9061) Illumina HiSeq 2000	(Graveley et al., 2011.4.13) (Graveley et al., 2011.4.13)
Data analysis	Bases were called using the most current version of the Illumina processing pipeline. The sequencing was performed using 76 nt reads on the AliX and 100 nt reads on the AliX appear to 100 nt reads on the AliX appear to 100 nt reads on the MaliX appear to 100 nt reads on the MaliX appear to 100 nt reads on the MaliX appear to 100 nt reads of 100 nt reads of 100 nt reads of 100 nt reads were the most of 100 nt reads were aligned using Bowlie v0.12.0 to a combined index of the genome and both annotated and predicted splice junctions. Paired-end alignments were further parsed using Spliced-Paired-end-Aligner (SPA, written by Michael Duff) to identify the optimal mapping location for matepairs.	(Graveley et al. 2011.4.13)
Additional data		
	More information is available under: modENCODE modMine: modENCODE_3207	
Associated files		
Additional sites		
Synonyms and Secon	dary IDs	
Reported As		
Symbol Synonym	Drosophila transcriptome tissue data (Graveley et al., 2011.4.	13)
	modENCODE_mRNA-Seq_tissues	
Secondary FlyBase IDs		
References (2)		
Personal communication to FlyBase	Graveley et al., 2011.4.13, The D. melanogaster transcriptome: modENCODE The D. melanogaster transcriptome: modENCODE RNA-Seq data for dissecte	
FlyBase analysis	FlyBase, 2011, Total read counts for modENCODE RNA-Seq data parental co Total read counts for modENCODE RNA-Seq data parental collections. [FBrf0.	

Dataset report (no parent report)



FB2014_01, released January 17th, 2014

Dataset report (no parent report)



Dataset for sequence features – list of members

GTCGGCAAT	COSTANGATA	GCCAAATA	ATATTATTATTGTTCAGATACTCAC FB2014_01, released January 17th, 2014							
AT ANTANTANA	TAL ATO COCIALAGOGO VALCACACA	AGT T ATCULA ACTTITY GTGCAACA	ACAGCOGGGGCAN	SE CITCATAGA		Datas	set mE	1_TFBS_H	ISA	
Home	Tools	Files	Species	Documents	Resour	ces News	Help	Archives	Jump	to Gene Go
								Help	Open All	Close All
General	Informatio	n								
Name		r	mE1_TFBS_H	SA		Species		D. melanogas	ster	
Dataset	type	٥	genomic sequ	ence feature		FlyBase ID		FBIc0000258		
Source a	and Conter	ıt								
Consists	s of		Genomic sequ sets.	ences identified by	y integrative	analysis of se	veral ChIF	odata (The m 2010)	nodENCODE Co.	nsortium,
Created	by									
Available	e from									
Strain										
Stage an	nd tissue									
Stage				Tissue/Position (i	ncluding su	bcellular local	ization)	Reference		
embryon	ic stage							(Negre et a	al., 2011)	
				Comment: TF b	inding profil	es used in this	analysis	were assayed at e	arly embryo stag	jes.
Cell Line)									
■ Rece	ent Updates	5								
■ Desc	ription and	Membe	rs							
Descript	tion		inding using l	ences identified as IOT spot analysis	(HSA); one	or many TFs n	nay bind ir	nagiven 2010)	nodENCODE Co.	nsortium,
		r	egion. A synth	esis of ChIP data	sets for 4 1 c	ifferent transci	ription fact	ors.		
Number	in collection	on 3	88562							
Commer	nt on numb on	er in								
Member	s		Download list	of the Large data	set metadata	a members				

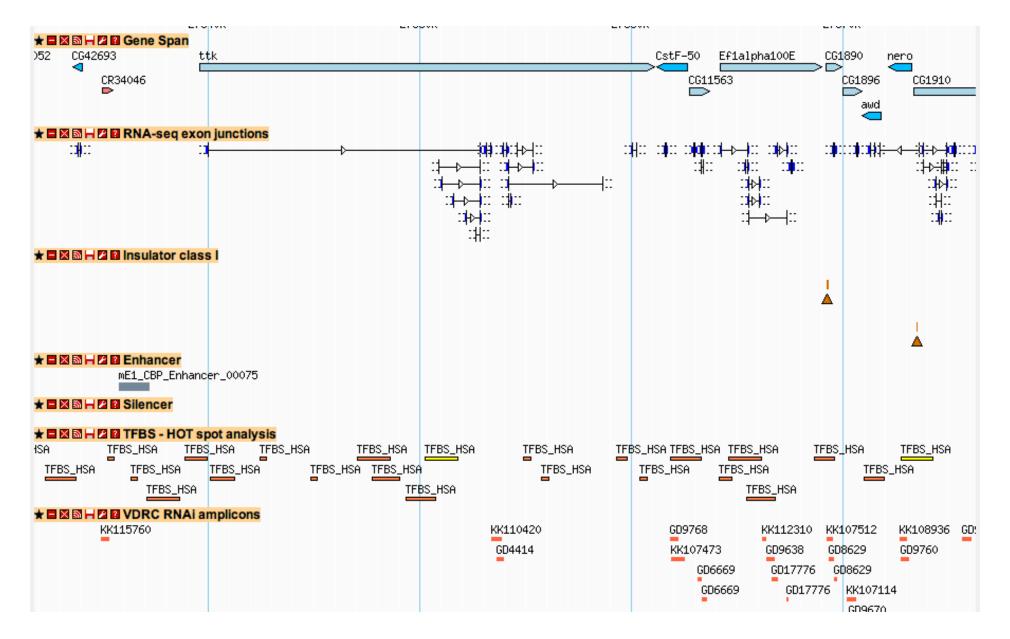
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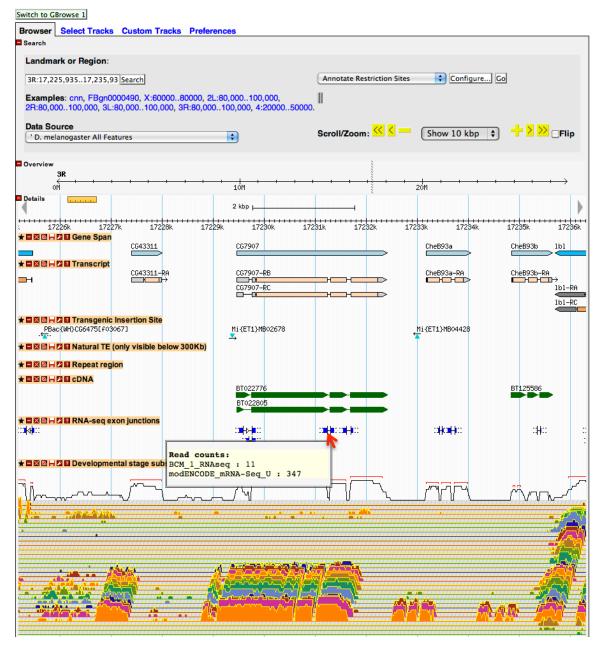
Sequence Features

What are sequence features?

- Defined sequence range
- Function



Sequence Features



Sequence Feature Glyph

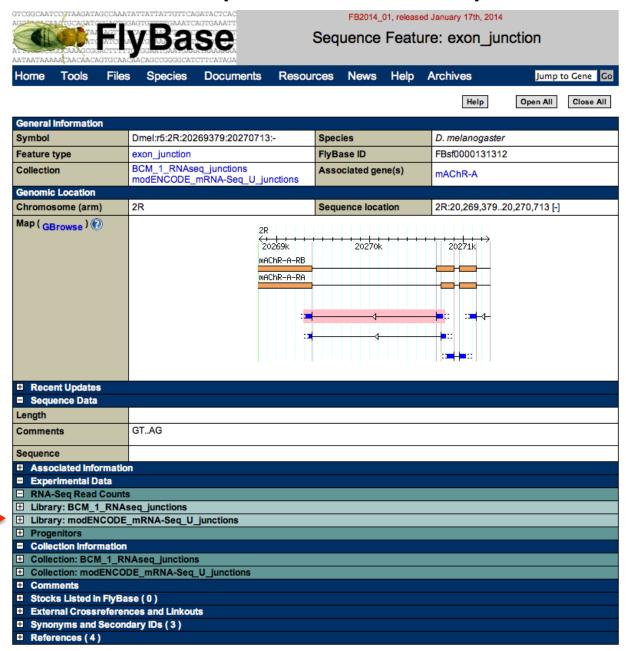
Hover: popup

Click: navigate to report

Example: RNA-Seq exon

junctions

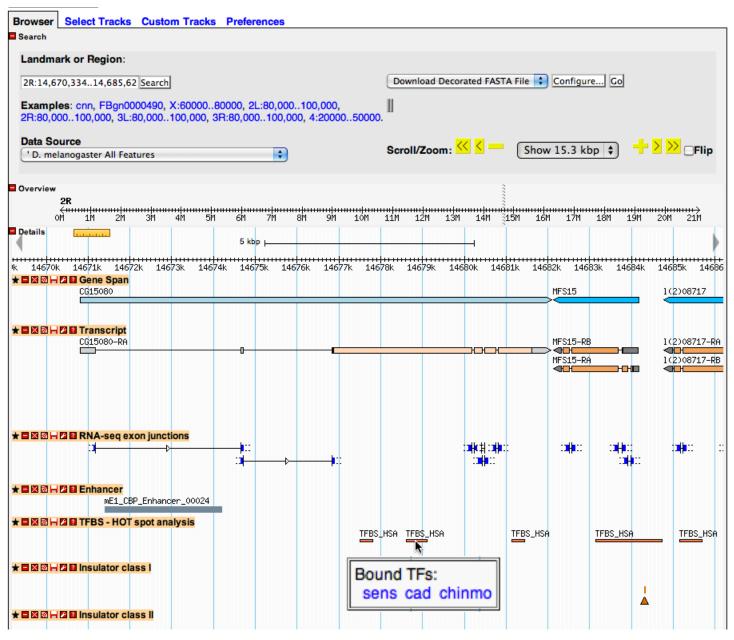
Sequence Feature reports: RNA-Seq Junctions

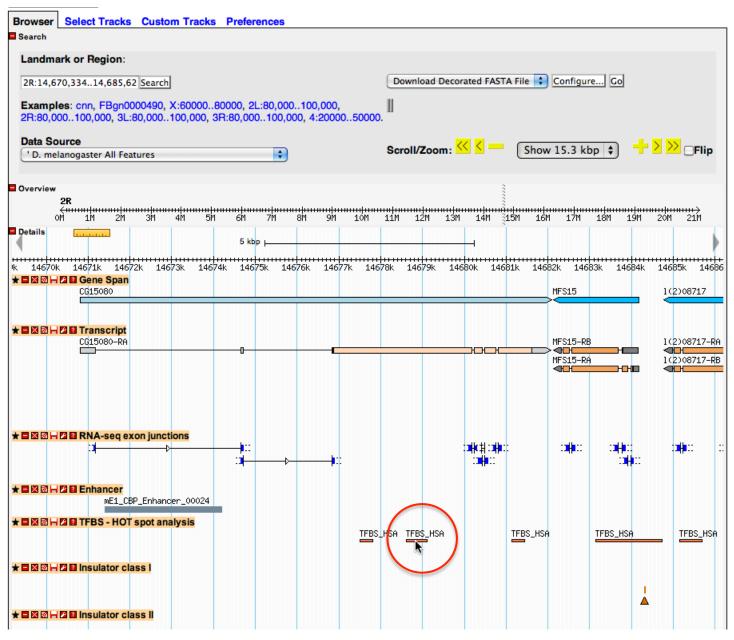


Sequence Feature reports: RNA-Seq Junctions

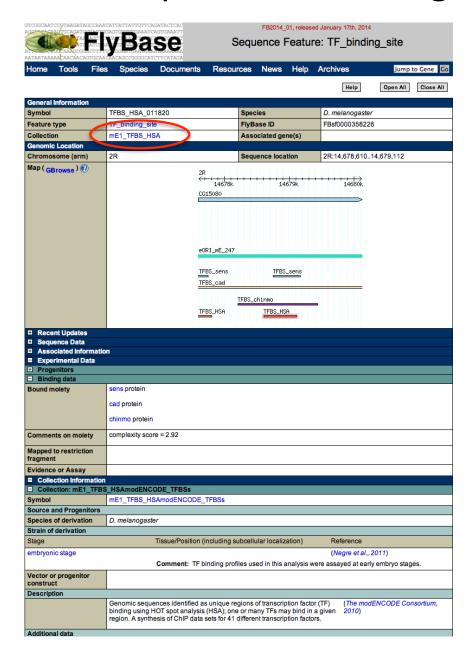
1	
embryonic stage 16 Comment:14-16 hr	7
embryonic stage 17(i) Comment:16-18 hr	23
embryonic stage 17(ii) Comment:18-20 hr	26
embryonic stage 17(iii) Comment:20-22 hr	23
embryonic stage 17(iv) Comment:22-24 hr	47
first instar larval stage	55
second instar larval stage	18
early third instar larval stage Comment:12 hr post-molt	1
third instar - uncleared gut stage Comment:indicated as puff stage 1-2	0

DE mDNA Con II impations		
DE_mRNA-Seq_U_junctions Expression stage(s)	Read Count	
embryonic stage 1 – 4	0	
Comment:0-2 hr	U	
embryonic stage 4 – 9	0	
Comment:2-4 hr		
embryonic stage 9 – 11 Comment: 4-6 hr	0	
embryonic stage 11 – 12	0	
Comment:6-8 hr	Ü	
embryonic stage 12 – 13	0	
Comment:8-10 hr		
embryonic stage 13 – 15 Comment:10-12 hr	0	
embryonic stage 15 16	0	
Comment:12-14 hr		
embryonic stage 16	7	
Comment:14-16 hr		
embryonic stage 17(i) Comment:16-18 hr	23	
embryonic stage 17(ii)	26	
Comment:18-20 hr		
embryonic stage 17(iii) Comment:20-22 hr	23	
	47	
embryonic stage 17(iv) Comment:22-24 hr	47	
first instar larval stage	55	
second instar larval stage	18	
early third instar larval stage	1	
Comment:12 hr post-molt	'	
third instar - uncleared gut stage	0	
Comment:indicated as puff stage 1-2		
third instar - partially cleared gut stage Comment:indicated as puff stage 3-6	1	
third instar - cleared gut stage	0	
Comment:indicated as puff stage 7-9		
prepupal stage P1	2	
pupal stage P5	4	
Comment:aged 12 hrs after collection as white prepupae		
pupal stage P6 Comment:aged 24 hrs after collection as white prepupae	3	
pharate adult stage P8	6	
Comment:aged 2 days after collection as white prepupae	-	
pharate adult stage P9 – P10 Comment:aged 3 days after collection as white prepupae	8	
	40	
pharate adult stage P15 Comment:aged 4 days after collection as white prepupae	13	
day 1 of adulthood female	6	
Comment:aged 1 day after eclosion		
day 1 of adulthood male Comment:aged 1 day after eclosion	6	
	2	
day 5 of adulthood female Comment:aged 5 days after eclosion	2	
day 5 of adulthood male	10	
Comment:aged 5 days after eclosion		
day 30 of adulthood female Comment:aged 30 days after eclosion	4	
day 30 of adulthood male	12	
Comment:aged 30 days after eclosion	12	
Total	277	
l .		









Routes to Dataset reports

- GBrowse help links
- Sequence feature reports
- Reference reports
- QuickSearch: Data Class "large dataset metadata"
- QueryBuilder: Large dataset metadata

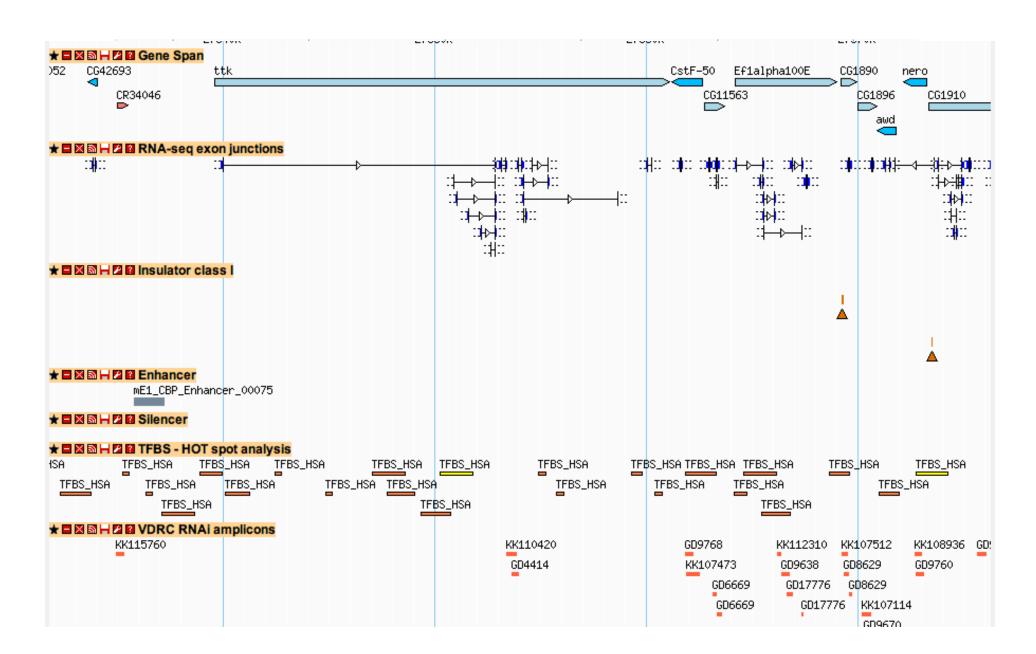
Routes to Sequence feature reports

- GBrowse links
- Download list from Dataset report
- FeatureMapper
- QuickSearch: Data Class "sequence features"
- QueryBuilder: Sequence Features
- Construct report (for RNAi amplicons)

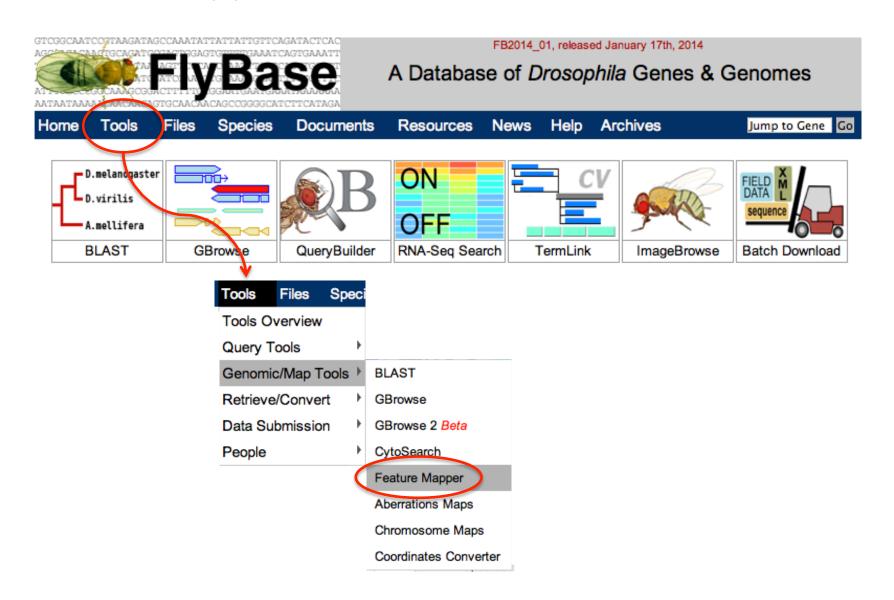
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FeatureMapper: find and retrieve features in region of interest



FeatureMapper: access via Tools menu



FeatureMapper input

Enter ID, Symbol, annotation ID or Sequence Region: 2R:14,668,80414,684,099 Sos arm		Sequence of the Landmark Include overlapping (not fully enclosed within query region) features Species: D. melanogaster		
Map Features:	■ Noncoding Features	☐ Mapped Mutations		
Genes mRNA (transcript) exon five_prime_UTR three_prime_UTR tRNA miRNA snRNA snoRNA CDS (polypeptide) Natural TE Aligned Evidence CDNA ESTs RNA-seq Exon Junctions Peptide Atlas peptides	Regulatory Regions Insulator class I Insulator class II Protein binding site Enhancers Silencers TFBS - HOT spot analysis TFBS - zinc finger domain TFBS - homeodomain TFBS - helix-loop-helix domain TFBS - BTB/POZ domain TFBS - other Origin of replication RNA Editing Sites Putative Brain Enhancers Microarray Features Affymetrix v1 Affymetrix v2	Transgene insertion sites Point Mutation Sequence Variant Uncharacterized Change in Seq. Aberration Junction Complex Substitution Indels Rescue Fragment RNAI Reagents and Data DGRC-1 amplicons DGRC-2 oligos DRSC RNAi amplicons VDRC RNAi amplicons TRIP RNAi amplicons BKNA RNAi amplicons HFA RNAi amplicons NIG-Fly RNAi amplicons		

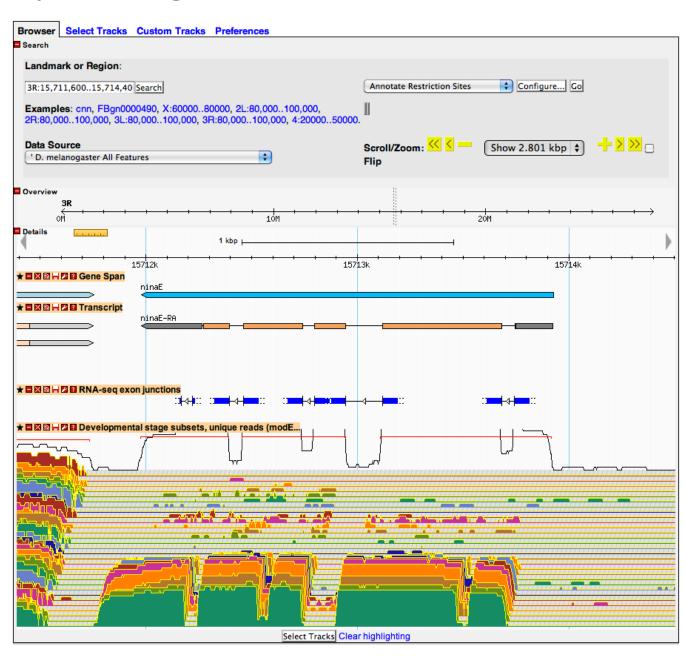
FeatureMapper output

2R:14,668,80414,	684 099			
ZR.14,000,00414,	,004,099			
All features (get GFF file)	←			
Insulators Class I	No features fou	und		
TFBS - HOT spot analysis	710 10010100 100			to HitList
Ti bo - not spot analysis	2D-14677507 146770	44	-> TF_binding_site:mE1_TFBS_HS	
			-> TF_binding_site:mE1_TFBS_HS	
			-> TF_binding_site:mE1_TFBS_HS	
DVA E 1111 O11			-> TF_binding_site:mE1_TFBS_HS	SA TFBS_HSA_011822
RNA Editing Sites	No features for	und		
Sos				
Sos				
All features get GFF file				
3	No footures for	und		
Insulators Class I	No features fou	ina		
TFBS - HOT spot analysis				(to HitList)
			-> TF_binding_site:mE1_TFBS_HS	
	2L:1381645013817860 -		-> TF_binding_site:mE1_TFBS_HS	
	2L:138180461381841	14	TF_binding_site:mE1_TFBS_HS	A TFBS_HSA_004502
	2L:138194751382085	55	-> TF_binding_site:mE1_TFBS_HS	A TFBS_HSA_004503
RNA Editing Sites	No features fou	ınd		
arm				
All features (get GFF file)				
Insulators Class I				to HitList
	X:17877611787771	>	insulator:Insulator_Class_I.mE01	Insulator_I_3962
	X:17945811794591	>	insulator:Insulator Class I.mE01	Insulator I 3963
TFBS - HOT spot analysis				(to HitList)
	X:17863921787874	>	TF_binding_site:mE1_TFBS_HSA	TFBS_HSA_031870
			TF binding site:mE1 TFBS HSA	TFBS HSA 031871
	1/ 1700101 1700011	-		TEDO 1101 0010T0

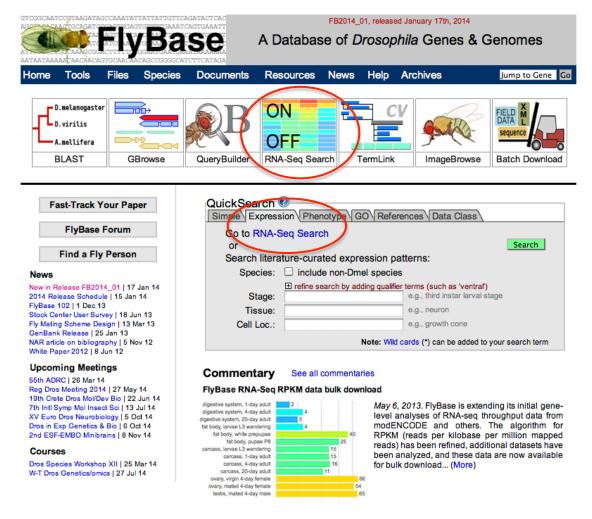
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RNA-Seq coverage data in GBrowse



Querying RNA-Seq expression data



FlyBase is supported by a grant from the National Human Genome Research Institute at the U.S. National Institutes of Health #P41 HG000739
Support is also provided by the British Medical Research Council, the Indiana Genomics Initiative, and the National Science Foundation
through XSEDE resources provided by Indiana University. Copyright Statement.

version FB2014_01, released January 17, 2014

Contact FlyBase

Cite FlyBase

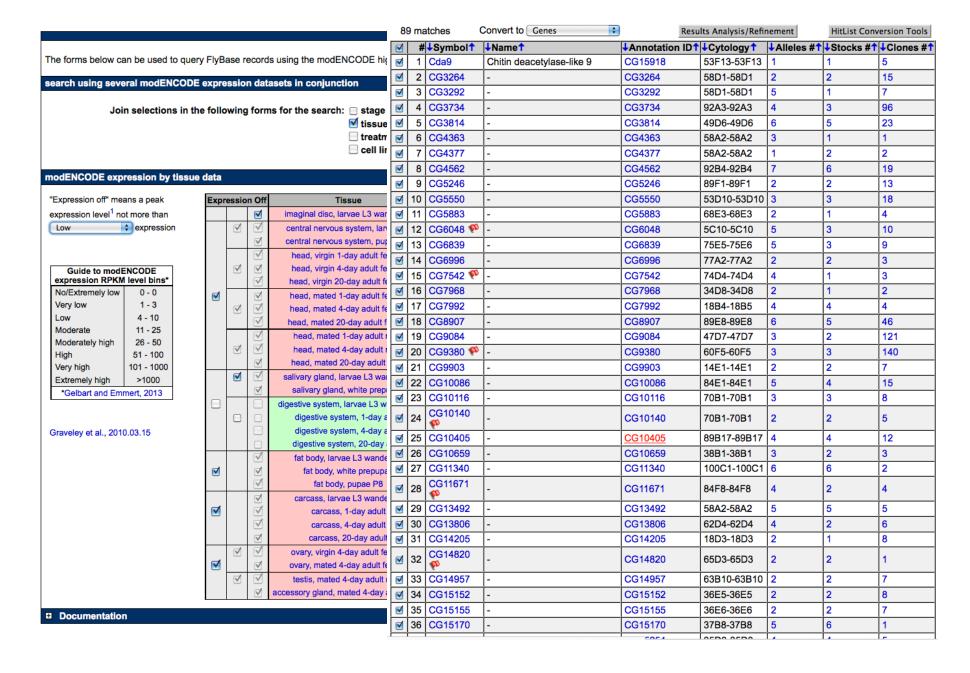
Site Map







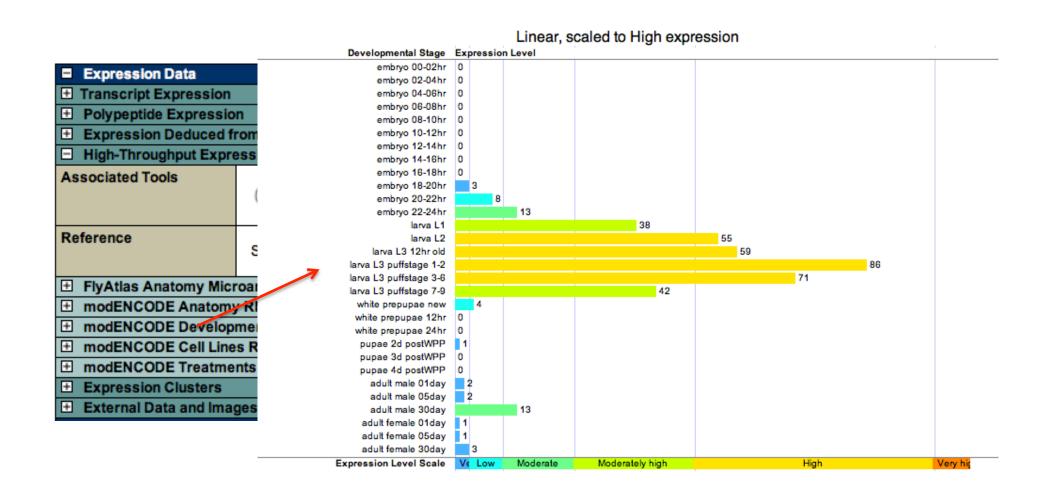
Querying RNA-Seq expression data



RNA-Seq coverage data: RPKM histograms



RNA-Seq coverage data: RPKM histograms



Release 6 is Coming!



- FlyBase is working with NCBI, BDGP and other major data producers to migrate all genome annotation data to Release 6.
- We have begun the migration process and expect it to be completed by the end of the summer of 2014.
- We will coordinate public migration to Release 6 with NCBI and other major genome archives starting in the summer of 2014.
- After Release 5.57 (May 2014), the next gene model annotation set will be on the Release 6 assembly.

References

Main modENCODE Publications:

Negre, et al. (2010). A comprehensive map of insulator elements for the Drosophila genome. PLoS Genet. 6(1): e1000814. (FBrf0209760)

modENCODE Consortium, et al. (2010). Identification of functional elements and regulatory circuits by Drosophila modENCODE. Science 330(6012): 1787--1797. (FBrf0212741)

Graveley, et al. (2011). The developmental transcriptome of Drosophila melanogaster. Nature 471(7339): 473--479. (FBrf0213330)

Negre, et al. (2011). A cis-regulatory map of the Drosophila genome. Nature 471(7339): 527--531. (FBrf0213303)

Kharchenko, et al. (2011). Comprehensive analysis of the chromatin landscape in Drosophila melanogaster. Nature 471(7339): 480--485. (FBrf0213294)

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Chung, et al. (2011). Computational and experimental identification of mirtrons in Drosophila melanogaster and Caenorhabditis elegans. Genome Res. 21(2): 286--300. (FBrf0213087)

Cherbas, et al. (2011). The transcriptional diversity of 25 Drosophila cell lines. Genome Res. 21(2): 301--314. (FBrf0213077)

Eaton, et al. (2011). Chromatin signatures of the Drosophila replication program. Genome Res. 21(2): 164--174. (FBrf0213065)

Riddle, et al. (2011). Plasticity in patterns of histone modifications and chromosomal proteins in Drosophila heterochromatin. Genome Res. 21(2): 147--163. (FBrf0213099)

Brooks, et al. (2011). Conservation of an RNA regulatory map between Drosophila and mammals. Genome Res. 21(2): 193--202. (FBrf0213079)

Smibert, et al. (2012). Global Patterns of Tissue-Specific Alternative Polyadenylation in Drosophila. Cell Rep. 1(3): 277--289. (FBrf0218523)

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Celniker, et al. (2009). Unlocking the secrets of the genome. Nature 459(7249): 927-930. (FBrf0215491)

Contrino, et al. (2012). modMine: flexible access to modENCODE data. Nucleic Acids Res. 40(1): D1082--D1088. (FBrf0217069)

Washington, et al. (2011). The modENCODE Data Coordination Center: lessons in harvesting comprehensive experimental details. Database (Oxford) 2011(): bar023. (FBrf0214707)