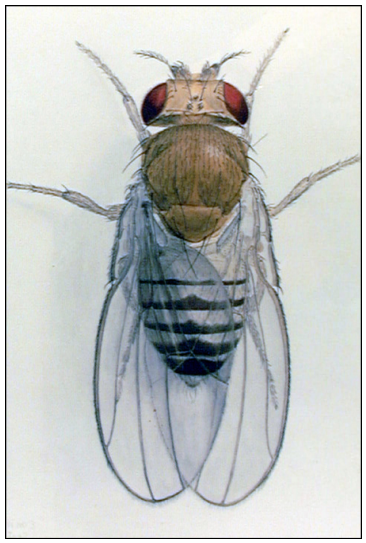
Comprehensive Identification of Worm and Fly Transcription Factor Binding Sites

Fly Transgenic Production and RNAi
Sue Celniker



1. Summary of BAC DNA Preparation
2. Summary of Transgenics Production
3. Transgenic Plan
1. RNAi Production and Plan
2. ChIP-peaks in oenocyte genes

The Transcription Factor Group Call
May 12, 2016

Injection Status as of 5/12/2016

Stabs sent	385 (301 < 35kb and 84 > 60kb)	
Transformed BACs	256	
Small BACs	229	
Large BACs	27	(23 by BDGP, 4 by Genetivision)

33 new lines since last call:	31 small BACs
	2 large BACs

TOTAL TFs in list	708
Completed prior effort	106
Completed this grant	256
TFs still to do	346

New Small BACs into AttP40 on 2nd chromosome (31)

bigmax
D
Dll
ERR
E(spl)m5
Fer2
Fer3
MBD-R2
pfk
phol
sqz
trem
CG1233
CG2199
CG2678
CG5180
CG6791
CG7271
CG7691
CG8216
CG8281
CG8301
CG9797
CG13296
CG13894
CG14711
CG14962
CG15514
CG17802
CG18764
CG31365

New Large BACs into AttP40 on 2nd chromosome (2)

lab
C15

Lines shipped to Chicago

Year 0		Year 1		Year 2		Year 3		Year 4	
		Q1 2013	0	Q1 2014	10	Q1 2015	20	Q1 2016	34
		Q2 2013	15	Q2 2014	16	Q2 2015	26	Q2 2016	est 35
		Q3 2013	21	Q3 2014	12	Q3 2015	34	Q3 2016	
Q4 2012	3	Q4 2013	0	Q4 2014	10	Q4 2015	16	Q4 2016	
TOTALS:	3		36		48		96		140

Lines shipped to Chicago

Year 0		Year 1		Year 2		Year 3		Year 4	
		Q1 2013	0	Q1 2014	10	Q1 2015	20	Q1 2016	34
		Q2 2013	15	Q2 2014	16	Q2 2015	26	Q2 2016	36
		Q3 2013	21	Q3 2014	12	Q3 2015	34	Q3 2016	35
Q4 2012	3	Q4 2013	0	Q4 2014	10	Q4 2015	16	Q4 2016	35
TOTALS:	3		36		48		96		140

At 35 per quarter, achieve 400 lines shipped around July 1, 2017.

Lines shipped to Chicago

Year 0		Year 1		Year 2		Year 3		Year 4	
		Q1 2013	0	Q1 2014	10	Q1 2015	20	Q1 2016	34
		Q2 2013	15	Q2 2014	16	Q2 2015	26	Q2 2016	36
		Q3 2013	21	Q3 2014	12	Q3 2015	34	Q3 2016	35
Q4 2012	3	Q4 2013	0	Q4 2014	10	Q4 2015	16	Q4 2016	35
TOTALS:	3		36		48		96		140

We can make about 140 more lines by July 21, 2017.

We have 75 small BACs in-house awaiting transformation. If we do 20 more large BACs, that leaves room for another 45 small BACs.

How to prioritize large BACs?

Small BACs ready to go into AttP40 on 2nd chromosome

D1
fd64A
pzg
Spps
Sry-Beta
tap
Trl-RA, RF
zen
zen2
CG4328
CG4936
CG6254
CG6813
CG6854
CG6905
CG7046
CG7056
CG9705
CG10267
CG10669
CG14655
CG15696
CG17186
CG18599

Small BACs ready to go into KV33 on 3rd chromosome

Br140
CoRest
Dek
dmrt11E
Dp
E2f2
ewg
fd59A
fd102c
gsb-n
Hand
Hmr
Lhr
MESR4
PHDP
Oli
schlank
Ssrp
Su(var)2-HP2
Su(var)2-10 (4 isoforms)
wek
zf30C
CG1529
CG1603
CG1621
CG2202
CG2875
CG2889
CG3407
CG4707
CG7101
CG7745
CG8290
CG8944 (2nd isoform)
CG9437
CG10209
CG10431
CG11906
CG12219
CG12299
CG12391
CG12659
CG12769
CG13204
CG13424
CG17385
CG32778
CG34367

Large BACs ready to go into AttP40 on 2nd chromosome

Abd-A (2 isoforms)
bap
Blimp1
croc
cwo
dan
dmrt99B
Doc2
E(spl)mdelta
exex
ftz
gl
Jarid 2
kni
mirr
mod(mdg) 5 isoforms
nerfin-1
nerfin-2
Poxm
ro
scrt
Sox100B
vvl
CG11560
CG12605
CG12768
CG16779
CG17806
CG31224

Large BACs ready to go into KV33 on 3rd chromosome

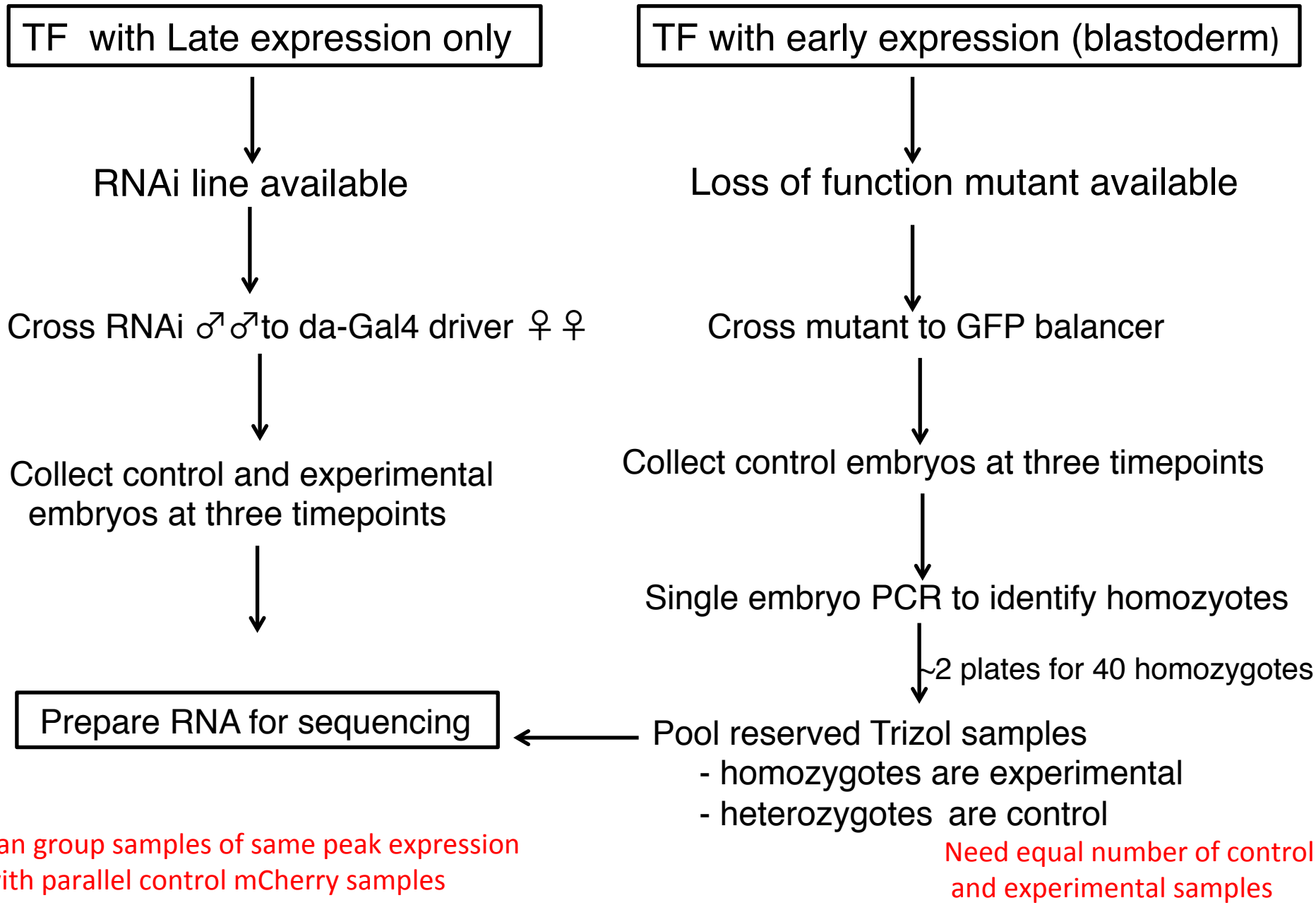
**ATbp*
**B-H2*
**Bap170*
**Dref*
**Optix*
**peb*
**salm*
**CG11085*

ac
Bsg25A
gem
Gsc
H15
Oaz
Poxn
Pph13
rib

RunxA
Set2
slp1
so
sob
wor
CG5953
CG10348
CG17612

* Transformant line inserted ectopically

Two Strategies for fly TF knockdown



Fly TFs for single embryo mutant selection

fly TF	worm ortholog	worm sequencing status	fly sequencing status	strategy
ems	ceh-2	low depth data	making RNA	single embryo genotyping
run	rnt-1	high depth data	GFP stock ready	single embryo genotyping
pros	ceh-26	bad deletion strain	GFP stock ready	single embryo genotyping
shn	sma-9	studied previously	crossing mutant to GFP balancer	single embryo genotyping
Antp	mab-5	high depth data	crossing mutant to GFP balancer	single embryo genotyping
pdm2	ceh-18	in progress	crossing mutant to GFP balancer	single embryo genotyping
Lim1	mec-3	low depth data	crossing mutant to GFP balancer	single embryo genotyping
btd	sptf-2	deletion strain order list	crossing mutant to GFP balancer	single embryo genotyping
ftz	no match	N/A	mutant at BDGP	single embryo genotyping
hb	hbl-1	not on worm lists?	mutant at BDGP	single embryo genotyping

Keep these in the pipeline but accelerate RNAi plan for late expressed TFs

Priority List for next 75-100 Fly TF knockdown targets

- TFs with patterned expression in embryos
    - where expression is only late, and RNAi line available
    - where expresssion is early, and loss of function mutant is available
- TFs with ChIP data available or tagged line made
- Fly TFs with worm orthologs
- Prioritize TFs expressed in selected organ systems?
- Prioritize uncharacterized TFs?

Revised plan for RNAi knockdown of TFs with late expression

Group by peak expression for more efficient use of controls and Illumina kits

Fly TF	worm ortholog	worm sequencing status	RNAi line	fly sequencing status	peak expression	estimated RNA ship date	number of samples including controls
twi	hlh-8	no deletion strain	HMS01317	sequenced			
nau	hlh-1	studied previously	HMC02974	sequenced		**7/24/15**	
E5	ceh-2	low depth data	HMJ21631	sequenced			
sens-2	pag-3	in progress	HMS01394	waiting for new kit		**shippped 3/24/16**	12
Hr51	fax-1	high depth data	HMS01951	setting up RNAi cross	14-16 h	**5/24/16**	12
scrt	ces-1	low depth data	HMC03996	expanding RNAi line	10-12h	**6/14/16**	18
Blimp-1	blmp-1	high depth data	HMC04792	expanding new RNAi line	10-12h	6/14/16	
scro	ceh-24	4x backcrossed	HMS00828	expanding RNAi line	12-14 h	**6/28/16**	24
onecut	ceh-48		HMS01438	RNAi line ordered	12-14 h	6/28/16	
CG9876			HMC03056	RNAi line ordered	12-14 h	6/28/16	
ss	ahr-1		HMS00296	RNAi line ordered	12-14h		
Ets65A	ast-1		HMS02246	on order list	12-14h		
AP-2	aptf-1		HMS02159	RNAi line ordered	10-12h	**7/5/16**	24
caup			HMS02716	on order list	10-12h		
fd59A	unc-130		HMC03155	on order list	10-12h		
CG33557	hlh-16		HMC03056	RNAi line ordered	6-8h		
dmrt99B	dmd-5		HMC03088	RNAi line ordered	6-8h		
pb			HMC03065	on order list	8-10h		
esn	prkl-1		HMS01360	in order list	14-16h		

Most efficient: Group three TFs (3 x 6 samples) with matching mCherry RNAi control for 24 samples per group. Two groups of 24 will use one Illumina kit.

mCherry RNAi control experiments

Control embryos for RNAi experiments:

- Expresses dsRNA for RNAi of mCherry under UAS control
- Activates RNAi system, but against a non-fly gene
- Tried first with *sens-2* RNAi experiments

$$\frac{\text{da-Gal4; da-Gal4}}{\text{da-Gal4; da Gal4}}\ ♀♀ \quad \text{x} \quad \frac{\text{UAS-}\textit{mCherry}\text{ RNAi}}{\text{UAS-}\textit{mCherry}\text{ RNAi}}\ ♂♂$$

For *sens-2* experiment:

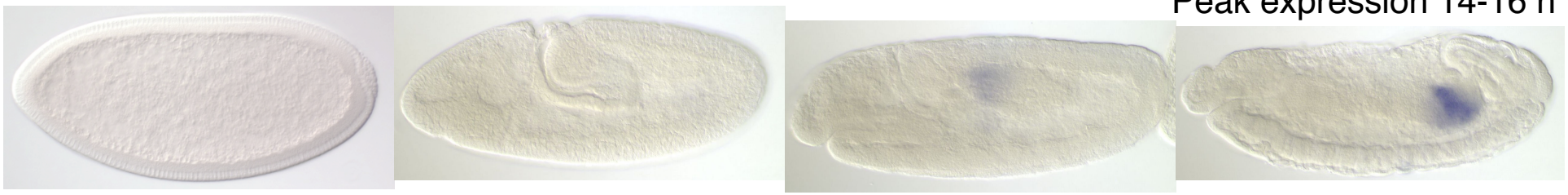
Three timepoints, 0-1.5, 14-16h (peak expression for target TF), 16-18 h

Early and late timepoints always the same, peak time point will vary with target TF

UAS-*mCherry* RNAi = y1 sc* v1; P{VALIUM20-mCherry}attP2

sens-2 knockdown by RNAi

Peak expression 14-16 h



St 4-6 St 9-10 St 11-12 St 13-16

Expressed in subset of the midgut starting at stage 11

da-driven RNAi for RNA-seq:

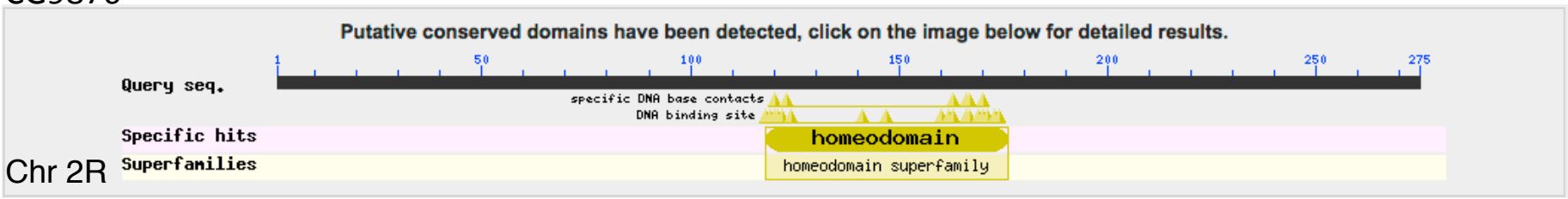
$$\frac{\text{da-Gal4; da-Gal4}}{\text{da-Gal4; da Gal4}}\ ♀♀ \quad X \quad \frac{\text{UAS-}\textit{sens-2}\text{ RNAi}}{\text{UAS-}\textit{sens-2}\text{ RNAi}}\ ♂♂$$

- all embryos have the same genotype: w*/y1, sc, v1; da-Gal4/+; daGal4/UAS sens-2 RNAi

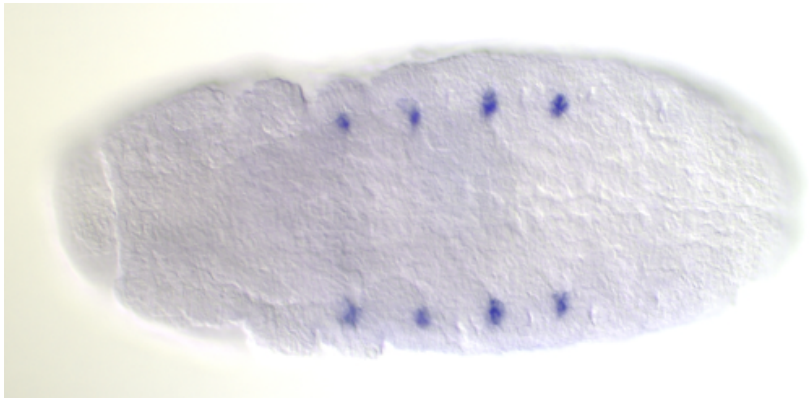
- >40% embryonic lethality
- of surviving embryos, 100% die before eclosing

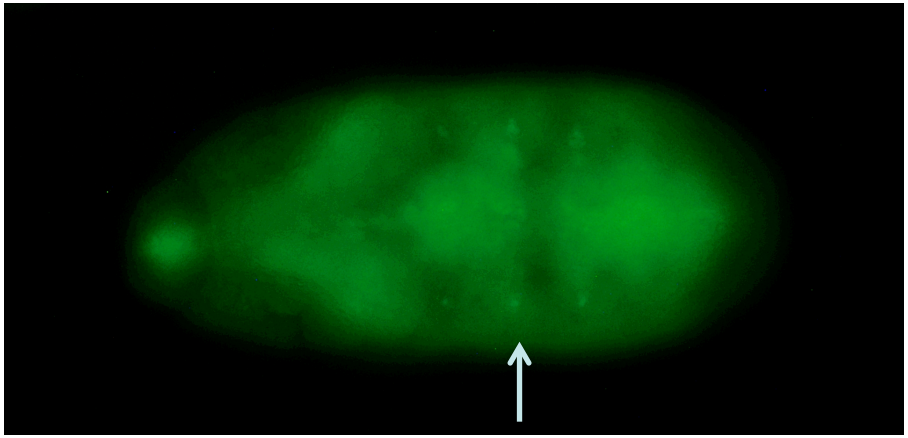
CG9876 wild type RNA *in situ* compared to GFP tag

CG9876



Chr 2R



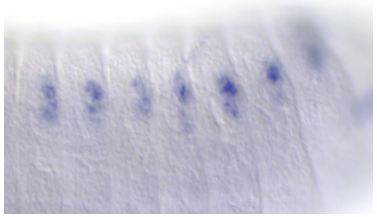


St 11 dorsal

Oenocyte precursers (ectodermal origin)

Genes with expression restricted to oenocytes

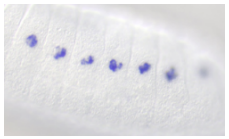
CG9876



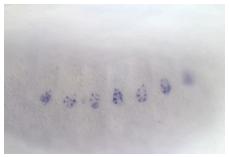
Transcription factor

Cpr

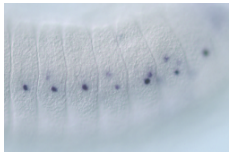
Cyp4g1



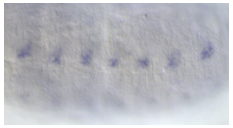
desat1



e

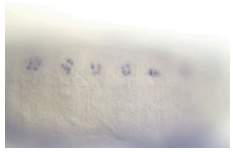


Hsp83

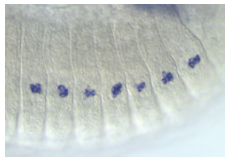


LpR1

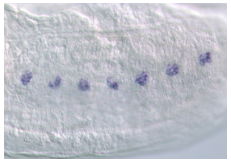
LpR2



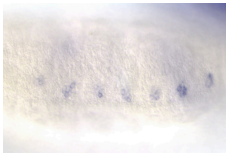
CG6921



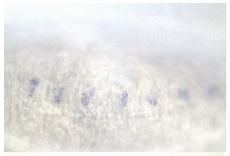
CG9459



CG12428

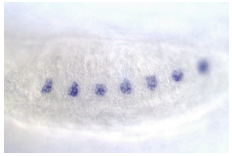


CG14615

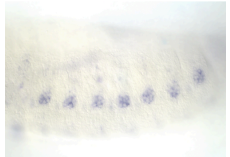


CG17562

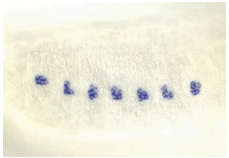
CG18031



CG18410



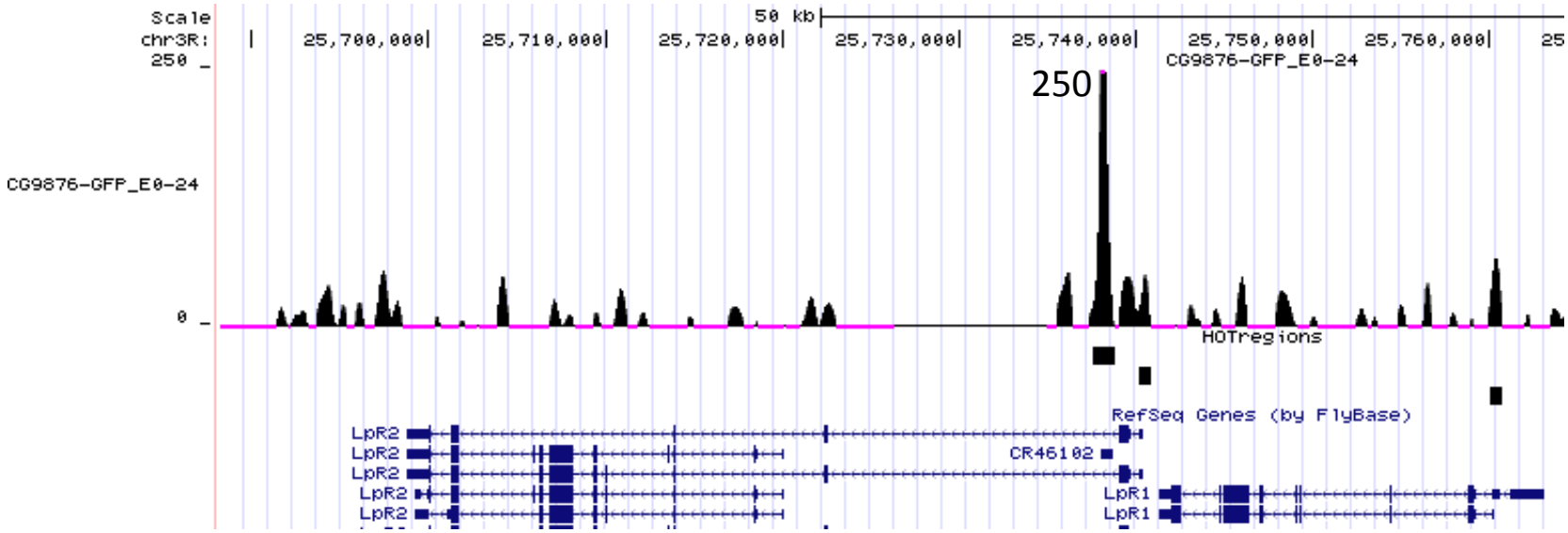
CG18609



ChIP peaks for oenocyte Transcription Factor CG9876

LpR2

LpR1



LpR2 *LpR1*

ChIP peaks for oenocyte Transcription Factor CG9876

ebony