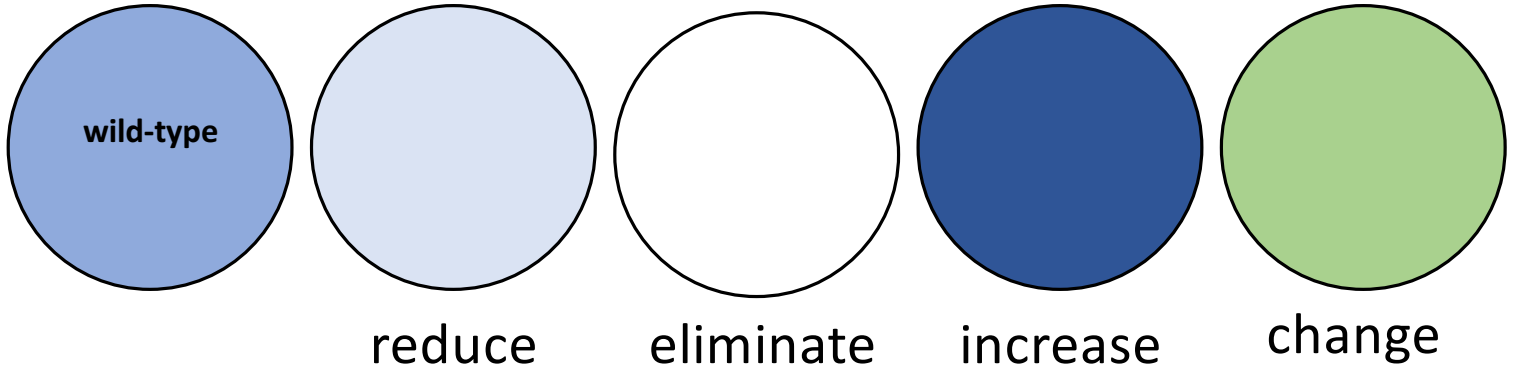
Drosophila research resources for you! at the DRSC & TRiP

Stephanie Mohr, PhD
Director of DRSC/TRiP Functional Genomics Resources
Laboratory of Prof. Norbert Perrimon
Harvard Medical School
http://fgr.hms.harvard.edu/

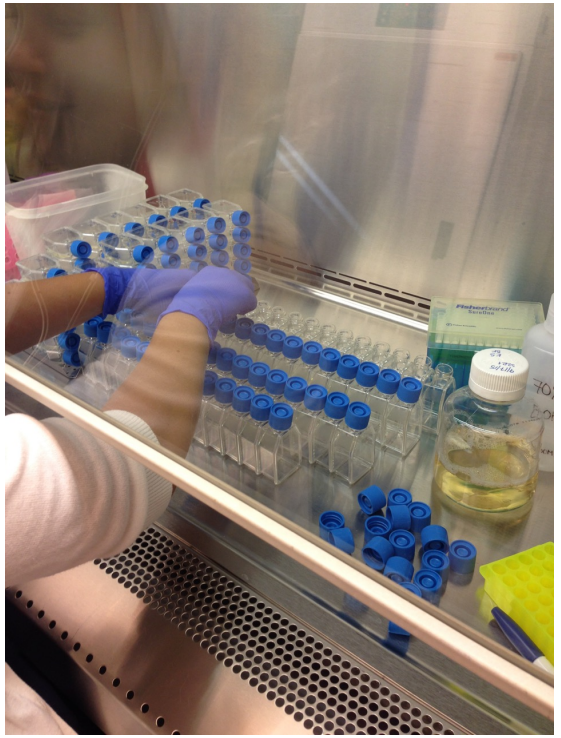
the ideal molecular genetic toolbox:



- for any cell type or tissue
- in a targeted, gene-specific manner
- at high-throughput scale

DRSC & TRiP

- Cell-based RNAi screening and libraries since 2003
- TRiP RNAi fly stocks since 2008
- Now & New: CRISPR technologies
- Growing suite of bioinformatics tools (find, analyze, validate)
- Welcome on-site visitors
- All reagents also provided for off-site screens or small-scale studies

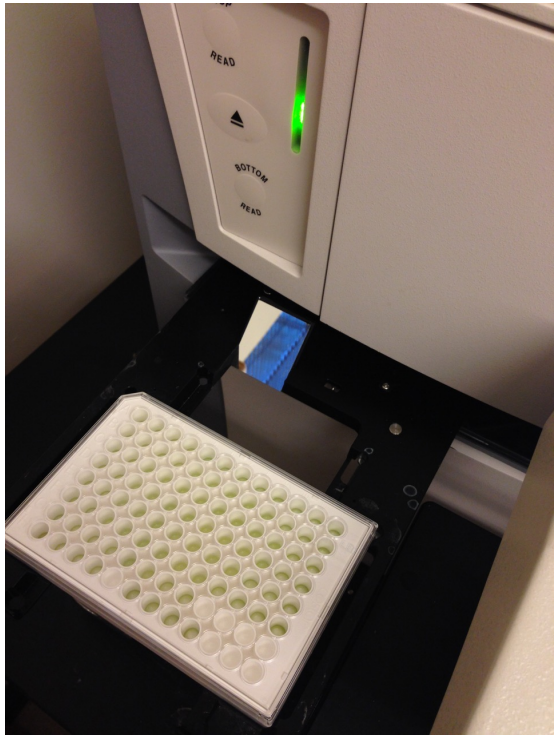


DRSC cell-based RNAi libraries

- **genome-wide screening library**
- **genome-wide validation library (PCR templates)**
- autophagy-related proteins
- GPCRs
- kinases & phosphatases
- membrane-bound organelle-related
- RNA-binding proteins
- transmembrane domain-containing
- transcription factors & DNA binding
- ubiquitin-related proteins
- **custom libraries (96-well format)**

cell-based screens

- arrayed RNAi screens
  - in CRISPR mutant backgrounds
  - interrogating synthetic interactions
  - high-content imaging
- pooled format screens
  - seeking collaborations, e.g. for selection-based pooled screens



screen assays @ the DRSC

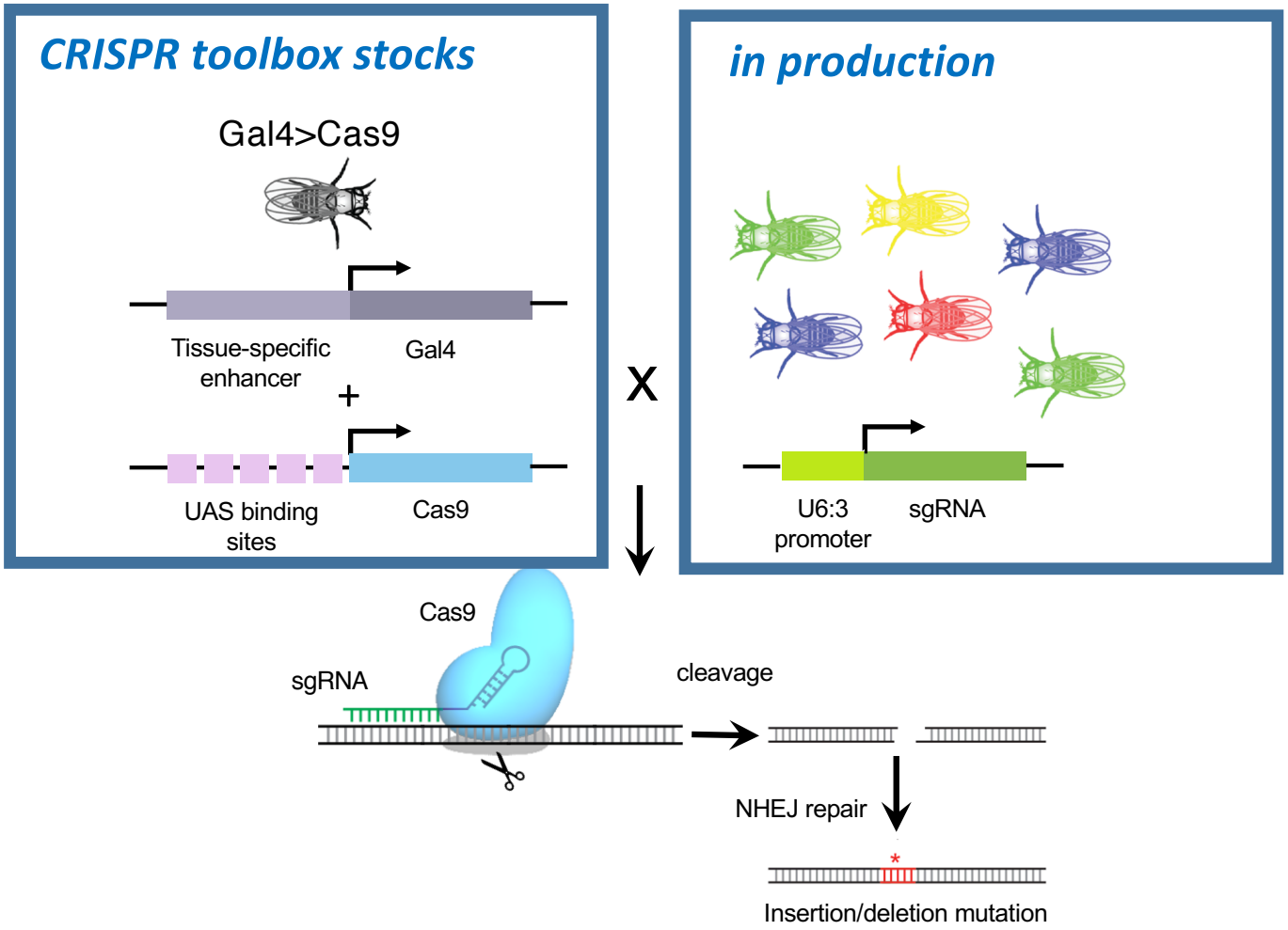
- Molecular Devices Spectramax Paradigm Fluorimeter & Luminometer (‘plate reader’)
- IN Cell 6000 automated confocal imaging system
  - slides, 6-well, 12-well, etc.
  - 96-well and 384-well
  - Kinedex plate handling robot

TRiP now and future

Jonathan Zirin

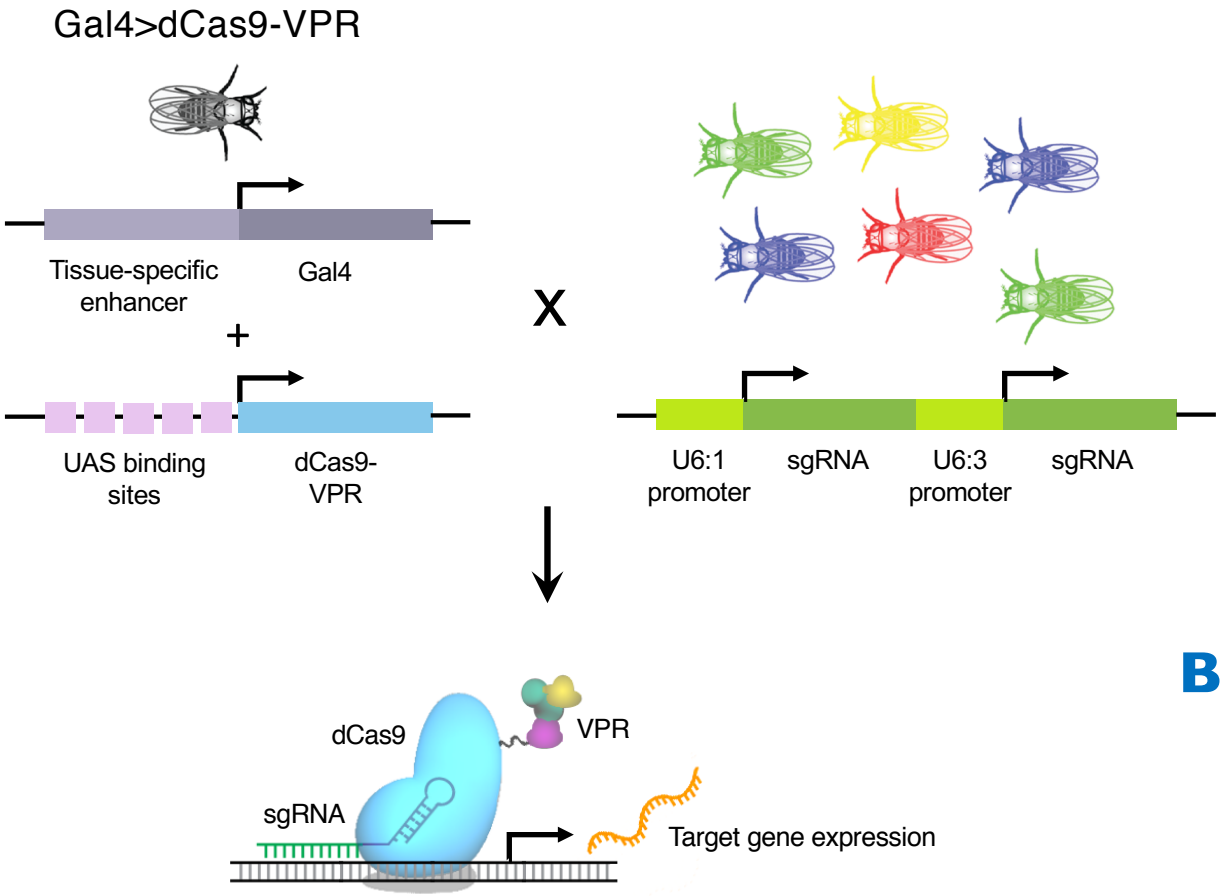
- ~10,000 UAS-RNAi fly stocks (Bloomington stock center)
- New: CRISPR stock production
  - knockout
  - activation
- CRIMIC project in collaboration with Hugo Bellen's lab (Baylor)
- CRISPR "toolbox" stocks

TRiP sgRNA fly stock collection for knockout



- A single gene is targeted by expression of one sgRNA from U6 promoter
- Stocks are made in the pCFD3 vector, developed by Fillip Port and colleagues
- Crossing sgRNA stocks to a Gal4 line expressing Cas9 induces Cas9, leading to mutation in many cells

TRiP sgRNA fly stock collection for transcriptional activation



- A single gene is targeted by two sgRNAs
- Stocks are made in the pCFD4 vector, developed by Fillip Port and colleagues

Ben Ewen-Campen

TRiP-CRISPR Toolbox stocks

dCas9-activator stocks (20 different Gal4 drivers)

- GAL4/UAS expression of Cas9 proteins with dead nuclease activity (dCas9), fused to VPR transcriptional activator (dCas9-a)
- used for gene activation in cells expressing gRNAs targeting the region upstream of the transcriptional start site (e.g. TEX lines)

dCas9-activator with tubGal80[ts] stocks (15 different Gal4 drivers)

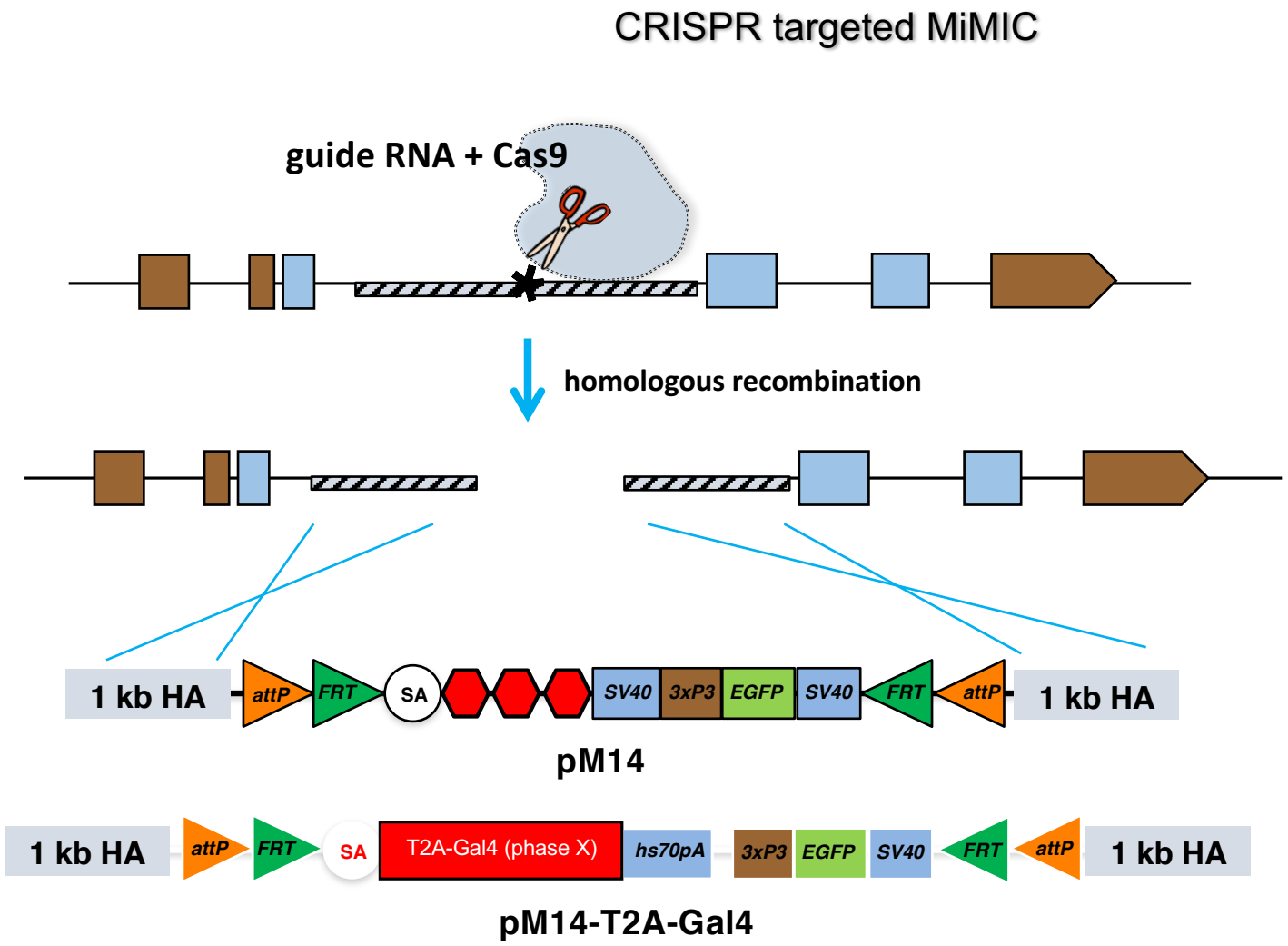
- GAL4/UAS combined with temperature-sensitive Gal80 (tubGal80[ts])
- allows greater control of spatial and temporal dCas9-a expression

Stocks for mosaic knock-outs (17 different Gal4 drivers)

- GAL4/UAS expression of wild type Cas9
- used for generating mutant mosaics in the soma in cells expressing sgRNAs targeting the coding region (e.g. TKO lines)

Stocks for germline mutants

- germline-specific nanos-GAL4/UAS expression of wild type Cas9
- used for generating small deletions and modifications in the germline in cells expressing sgRNAs targeting the coding region



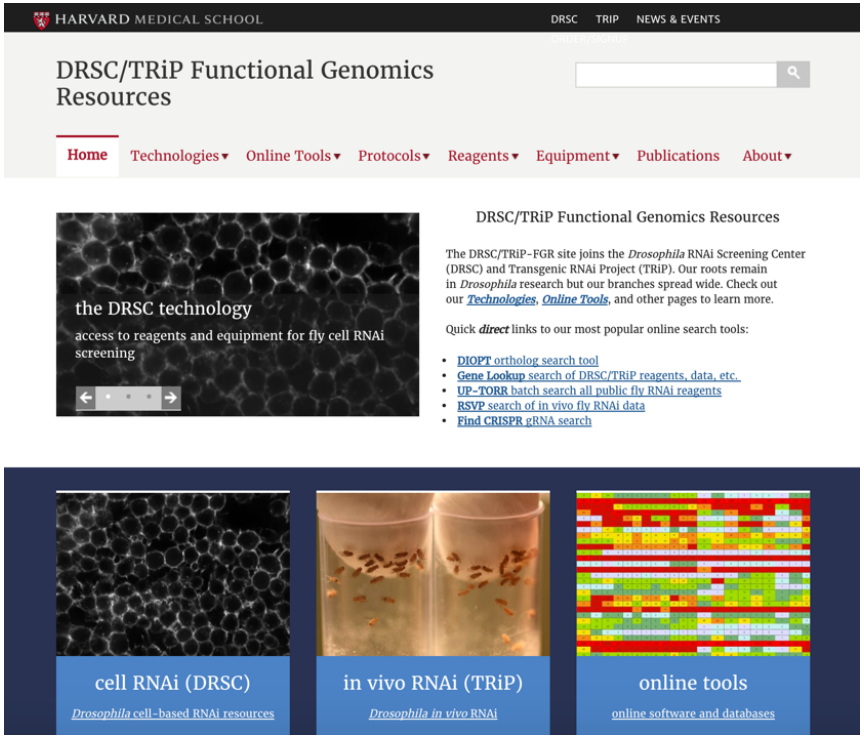
CRISPR MiMIC Integration Cassettes (CRIMICs)

- With CRISPR it is feasible to integrate SICs in user-defined locations rather than at random as is done with TEs
- Project to target genes that have the greatest utility for the fly community and human disease orthologs
- CRIMIC gene traps now contain T2A-Gal4, expressed under the control of endogenous gene regulatory elements
- As with MiMIC, CRIMIC permit protein tagging by RMCE
- CRiMIC/MIMIC gene and protein traps are available from the Gene Disruption Project (Bellen) and the BDSC

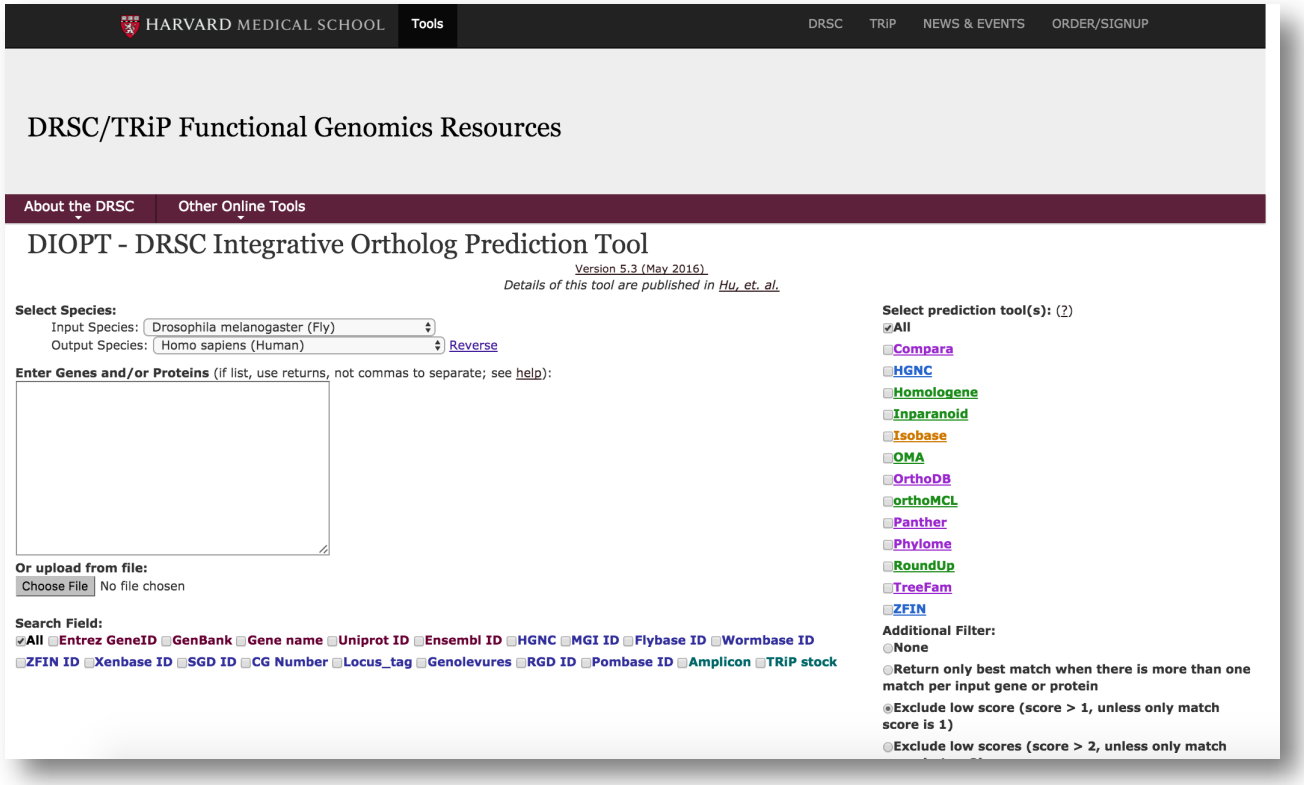
bioinformatics

Claire Hu

- DIOPT – ortholog prediction
- UP-TORR – identify RNAi reagents
  - Cell reagents – DRSC, DKFZ
  - In vivo fly stocks – VDRC, NIG-Japan, TRiP
- RSVP – view RNAi fly stock phenotypes
- FlyPrimerBank – qPCR primer designs
- Find CRISPRs – genome browse view of gRNA designs
- DGET – mine modENCODE and other RNA-seq data
- *and more!*



DIOPT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About the DRSC
Other Online Tools
DIOPT - DRSC Integrative Ortholog Prediction Tool
Version 5.3 (May 2016)
Details of this tool are published in Hu, et. al.
Select Species:
Input Species: Drosophila melanogaster (Fly)
Output Species: Homo sapiens (Human)
Reverse
Enter Genes and/or Proteins (if list, use returns, not commas to separate; see help):
Or upload from file:
Choose File
No file chosen
Search Field:
All Entrez GeneID GenBank Gene name Uniprot ID Ensembl ID HGNC MGI ID Flybase ID Wormbase ID
ZFIN ID Xenbase ID SGD ID CG Number Locus_tag Genolevures RGD ID Pombase ID Amplicon TRiP stock
Select prediction tool(s): (?)
All
Compara
HGNC
Homologene
Inparanoid
Isobase
OMA
OrthoDB
orthoMCL
Panther
Phylome
RoundUp
TreeFam
ZFIN
Additional Filter:
None
Return only best match when there is more than one match per input gene or protein
Exclude low score (score > 1, unless only match score is 1)
Exclude low scores (score > 2, unless only match

“all” search

DIOPT

Show Summary of Best Scores

Search Term	Fission yeast Gene ID	PomBase	Fissio yeas Symb	Species 2	Species 2 Gene ID	Species 2 Spe Gene L	Species 2 ene Name	Score	Weighted Score	Rank	Best Score?	Best Score Reverse Search?	Prediction Derived From	Alignment & Scores
ers1	2538690	SPCC1393.05	ers1					0	0.0	-		None		
wtf13	2538692	SPCC162.04c	wtf13					0	0.0	-		None		
emp24	2539350	SPCC24B10.17	emp2	Human	10959			8	7.888	High	Yes	Yes	Homologene, Inparanoid, OMA, orthoMCL, Phylome, RoundUp, TreeFam, Panther	View
emp24	2539350	SPCC24B10.17	emp2	Mouse	56334		2	6	5.973	High	Yes	Yes	Homologene, OMA, orthoMCL, RoundUp, TreeFam, Panther	View
emp24	2539350	SPCC24B10.17	emp2	Mouse	00042343		98	4	3.918	Moderate	No	Yes	Inparanoid, OMA, orthoMCL, RoundUp	
emp24	2539350	SPCC24B10.17	emp2	Rat	65165		2	6	5.975	High	Yes	Yes	Homologene, Inparanoid, OMA, orthoMCL, TreeFam, Panther	View
emp24	2539350	SPCC24B10.17	emp2	Zebrafish	321550	ZDB-GENE-030131-269	tmed2	6	5.973	High	Yes	Yes	Homologene, OMA, orthoMCL, RoundUp, TreeFam, Panther	View
emp24	2539350	SPCC24B10.17	emp2	Western clawed frog	734103	XB-GENE-986353	tmed2	5	4.978	High	Yes	Yes	Homologene, Inparanoid, OMA, RoundUp, TreeFam	View
emp24	2539350	SPCC24B10.17	emp2	Fly	31382	FBgn0029709	CHOp24	7	6.878	High	Yes	Yes	Homologene, Inparanoid, orthoMCL, Phylome, RoundUp, TreeFam, Panther	View
emp24	2539350	SPCC24B10.17	emp2	Fly	37507	FBgn0034681	CG9308	5	4.875	Moderate	No	Yes	Inparanoid, orthoMCL, Phylome, TreeFam, Panther	View
emp24	2539350	SPCC24B10.17	emp2	Worm	179213	WBGene00004766	sel-9	6	6.018	High	Yes	Yes	Homologene, Inparanoid, OMA, orthoMCL, RoundUp, Panther	View
emp24	2539350	SPCC24B10.17	emp2	Yeast	852675	S000003168	EMP24	8	7.888	High	Yes	Yes	Homologene, Inparanoid, OMA, orthoMCL, Phylome, RoundUp, TreeFam, Panther	View
ubc12	2539416	SPCC777.10c	ubc1	Human	9040	12491	UBE2M	7	6.928	High	Yes	Yes	Homologene, Inparanoid, OMA, orthoMCL, Phylome, RoundUp, Panther	View

Search with human genes

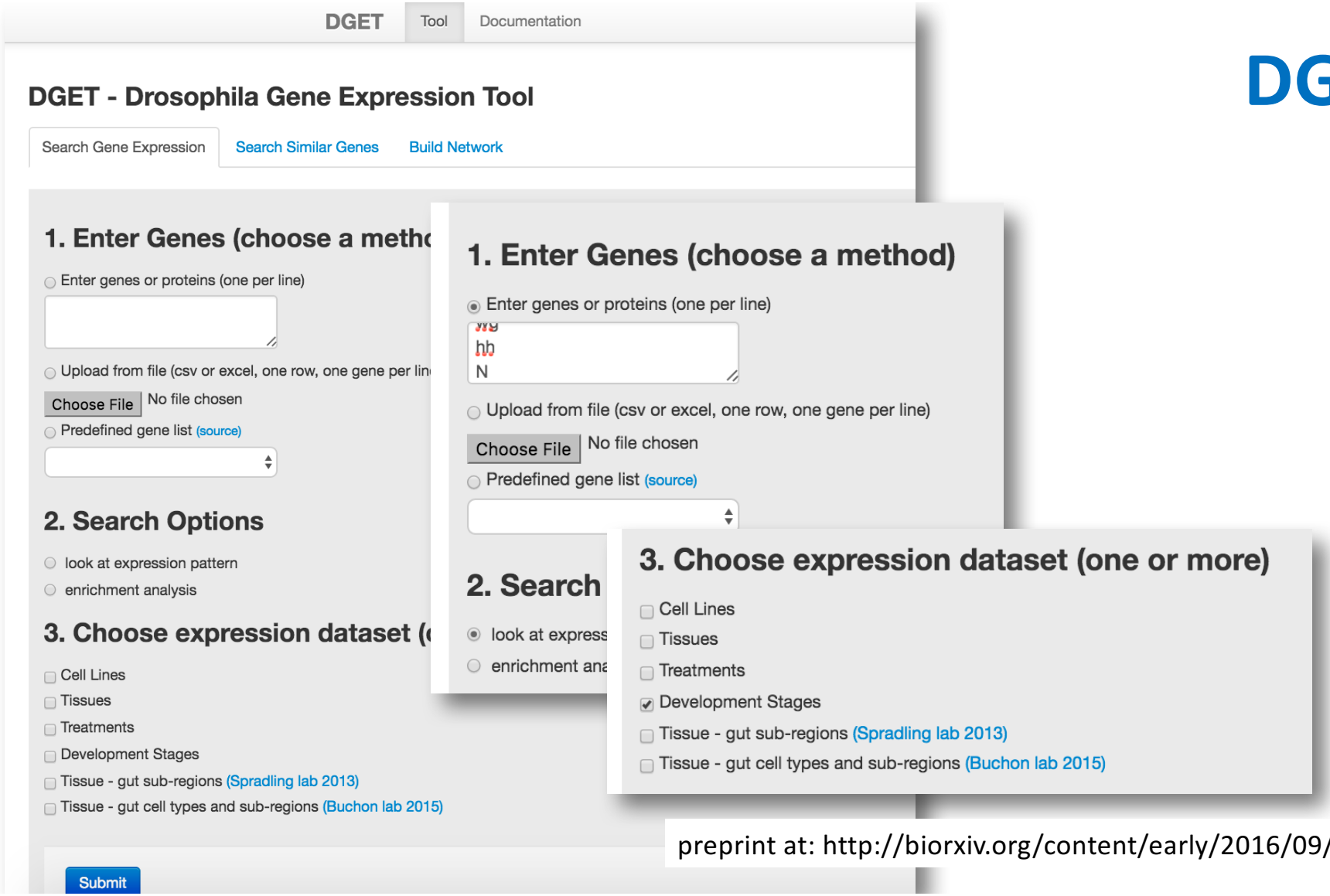
DIOPT

Search Term	Fission yeast	Budding yeast	Worm	Fly	Zebrafish	Frog	Rat	Mouse	Human
AGPAT2	slc1 (7 of 8)	SLC1 (7 of 10)	acl-2 (7 of 11)	CG3812 (7 of 11)	agpat2 (6 of 11)	agpat2 (4 of 8)	Agpat2 (6 of 8)	Agpat2 (10 of 12)	NA
DECR1	SPAC8E11.10,SPCC1739.08c (2 of 8)	SPS19 (3 of 10)	F53C11.3 (10 of 11)	CG18814,Pdh,CG4842 (2 of 11)	decr1 (10 of 11)	decr1 (5 of 8)	Decr1 (8 of 8)	Decr1 (12 of 12)	NA
DHCR24	----	----	F52H2.6 (11 of 11)	----	dhcr24 (8 of 11)	dhcr24 (5 of 8)	Dhcr24 (4 of 8)	Dhcr24 (11 of 12)	NA
BPGM	gpm1 (5 of 8)	GPM1 (6 of 10)	----	CG7059 (5 of 11)	bpgm (11 of 11)	bpgm (7 of 8)	Bpgm (6 of 8)	Bpgm (11 of 12)	NA
OAS1	----	----	----	----	----	----	Oas1a (6 of 8)	Oas1a (9 of 12)	NA

Search with fission yeast genes

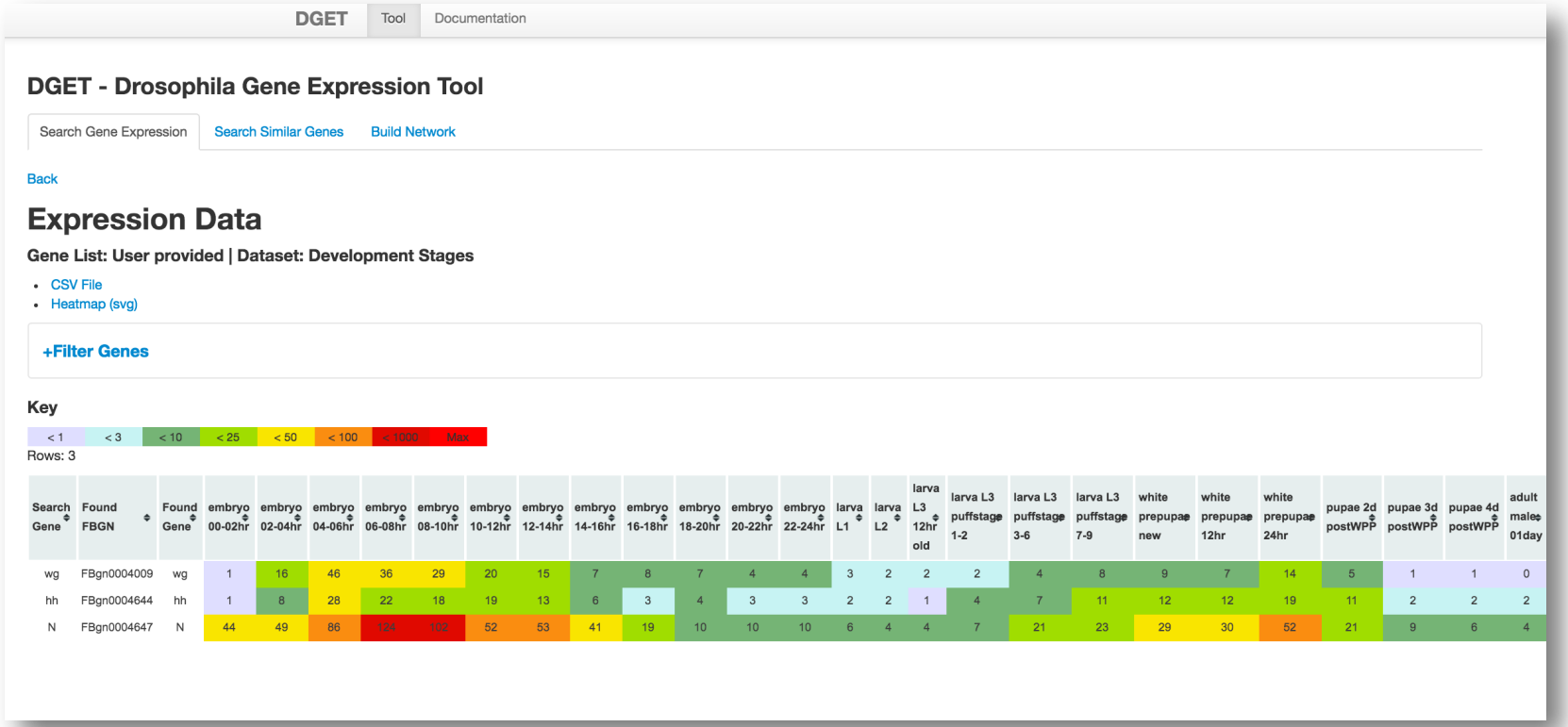
Search Term	Fission yeast	Budding yeast	Worm	Fly	Zebrafish	Frog	Rat	Mouse	Human
vps32	NA	SNF7 (8 of 8)	vps-32.2 (6 of 7)	shrb (8 of 8)	chmp4bb (6 of 7)	chmp4b (5 of 5)	Chmp4bl1 (6 of 6)	Chmp4b (7 of 7)	CHMP4B (8 of 8)
gtr2	NA	GTR2 (7 of 8)	ragc-1 (7 of 7)	RagC-D (8 of 8)	rragcb (6 of 7)	rragc (5 of 5)	Rragc (6 of 6)	Rragc (7 of 7)	RRAGC (8 of 8)
rps1602	NA	RPS16A (8 of 8)	rps-16 (6 of 7)	RpS16 (8 of 8)	rps16 (7 of 7)	rps16 (5 of 5)	Rps16 (6 of 6)	Rps16 (6 of 7)	RPS16 (8 of 8)
fma1	NA	MAP1 (8 of 8)	map-1 (7 of 7)	CG13630 (8 of 8)	metap1 (7 of 7)	metap1 (5 of 5)	Metap1 (6 of 6)	Metap1 (7 of 7)	METAP1 (8 of 8)
emp24	NA	EMP24 (8 of 8)	sel-9 (6 of 7)	CHOp24 (7 of 8)	tmed2 (6 of 7)	tmed2 (5 of 5)	Tmed2 (6 of 6)	Tmed2 (6 of 7)	TMED2 (8 of 8)
sec61	NA	SEC61 (8 of 8)	sec-61 (6 of 7)	Sec61alpha (7 of 8)	sec61a1l,sec61a1 (7 of 7)	sec61a1 (4 of 5)	Sec61a1 (6 of 6)	Sec61a1 (7 of 7)	SEC61A1 (8 of 8)
coq9	NA	COQ9 (7 of 8)	----	CG30493 (7 of 8)	coq9 (4 of 7)	coq9 (4 of 5)	Coq9 (5 of 6)	Coq9 (6 of 7)	COQ9 (7 of 8)
ubc11	NA	UBC11 (8 of 8)	----	vih (8 of 8)	ube2c (4 of 7)	ube2c (3 of 5)	Ube2c (5 of 6)	Ube2c (6 of 7)	UBE2C (7 of 8)
ubc12	NA	UBC12 (8 of 8)	ubc-12 (3 of 7)	Ubc12 (8 of 8)	ube2f (3 of 7)	ube2m (4 of 5)	Ube2m (4 of 6)	Ube2m (6 of 7)	UBE2M (7 of 8)
set11	NA	RKM2 (5 of 8)	set-29 (5 of 7)	CG33230 (5 of 8)	setd4 (4 of 7)	setd4 (2 of 5)	Setd4 (4 of 6)	Setd4 (6 of 7)	SETD4 (7 of 8)
glt1	NA	GLT1 (8 of 8)	W07E11.1 (7 of 7)	CG9674 (8 of 8)	----	----	----	----	----
mtf1	NA	MTF1 (7 of 8)	----	----	----	----	----	----	TFB1M (2 of 8)
ers1	NA	----	----	----	----	----	----	----	----
wtf13	NA	----	----	----	----	----	----	----	----

DGET



preprint at: http://biorxiv.org/content/early/2016/09/15/075358

DGET



DGET Tool Documentation

DGET - Drosophila Gene Expression Tool

Search Gene Expression | Search Similar Genes | Build Network

Back

Expression Data

Gene List: User provided | Dataset: Development Stages

- CSV File
- Heatmap (svg)

+Filter Genes

Key

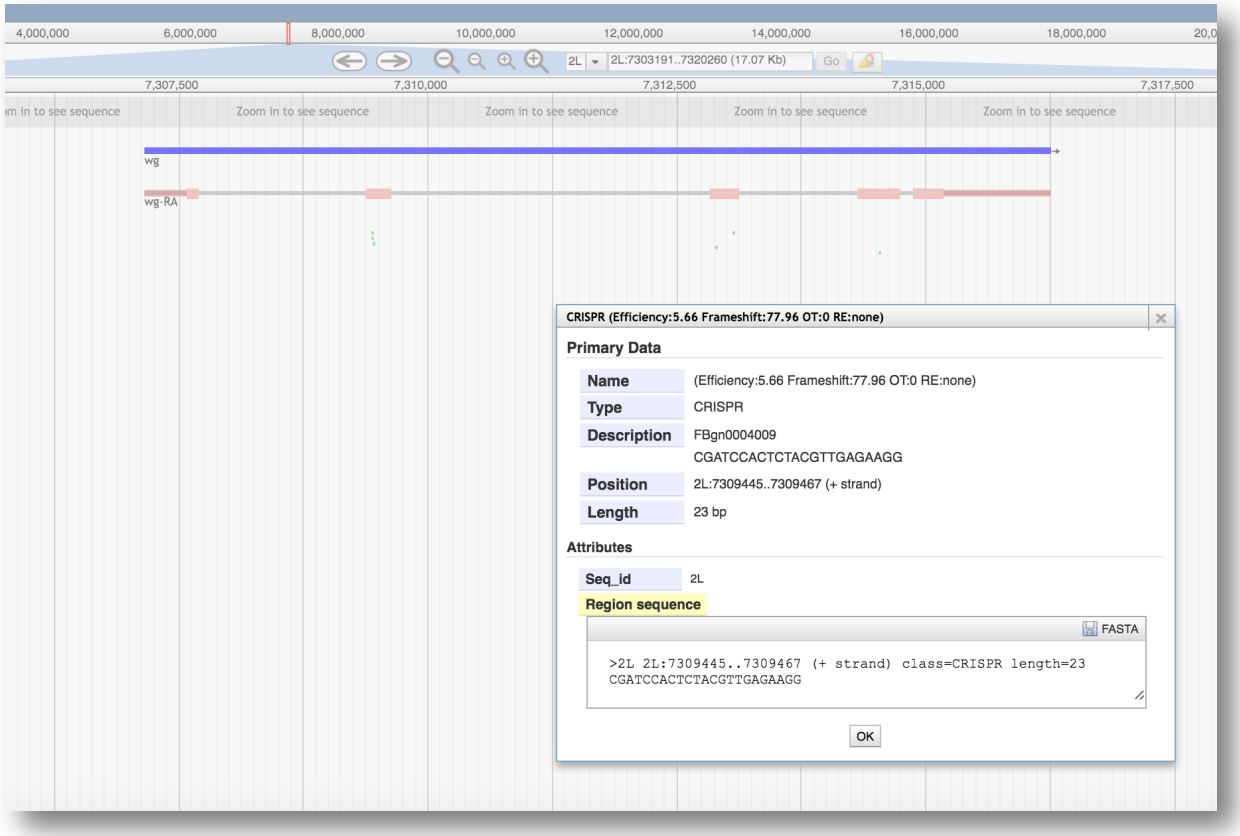
< 1 | < 3 | < 10 | < 25 | < 50 | < 100 | < 1000 | Max

Rows: 3

Search Gene	Found FBGN	Found Gene	embryo 00-02hr	embryo 02-04hr	embryo 04-06hr	embryo 06-08hr	embryo 08-10hr	embryo 10-12hr	embryo 12-14hr	embryo 14-16hr	embryo 16-18hr	embryo 18-20hr	embryo 20-22hr	embryo 22-24hr	larva L1	larva L2	larva L3 12hr old	larva L3 puffstage 1-2	larva L3 puffstage 3-6	larva L3 puffstage 7-9	white prepupae new	white prepupae 12hr	white prepupae 24hr	pupae 2d postWPP	pupae 3d postWPP	pupae 4d postWPP	adult male 01day
wg	FBgn0004009	wg	1	16	46	36	29	20	15	7	8	7	4	4	3	2	2	2	4	8	9	7	14	5	1	1	0
hh	FBgn0004644	hh	1	8	28	22	18	19	13	6	3	4	3	3	2	2	1	4	7	11	12	12	19	11	2	2	2
N	FBgn0004647	N	44	49	86	124	102	52	53	41	19	10	10	10	6	4	4	7	21	23	29	30	52	21	9	6	4

preprint at: http://biorxiv.org/content/early/2016/09/15/075358

Find CRISPRs



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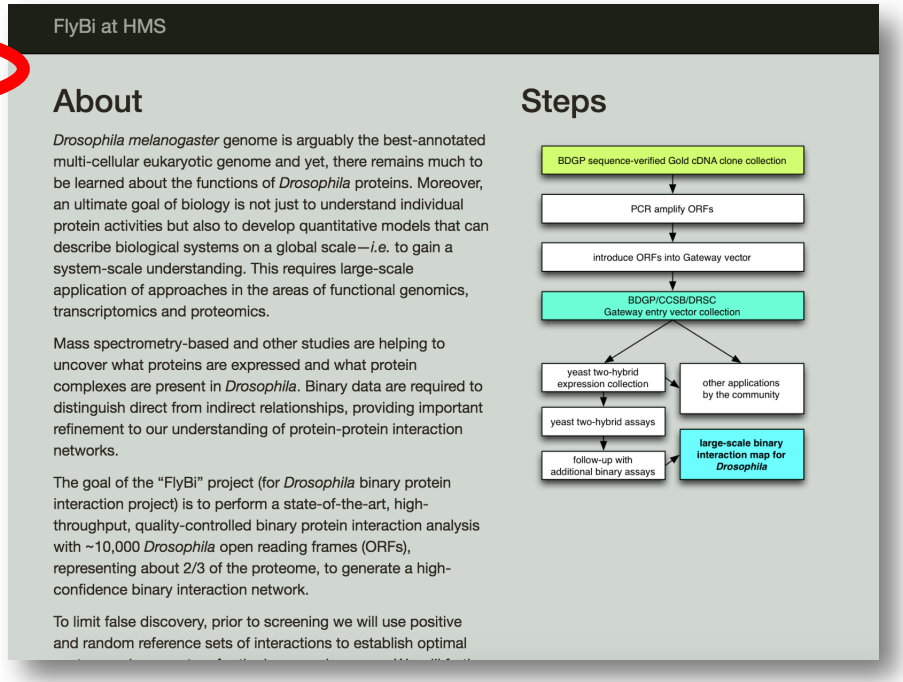
Drosophila Binary Interaction Project: "FlyBi"

- Collaboration with the Celniker and Vidal labs
- Resource of **~10,000 Gateway ORF clones**
- available now from:
  - DF/HCC DNA Resource Core (Boston, MA)
  - DNASU (Phoenix, AZ)
  - *Drosophila* Genome Resource Center (Bloomington, IN)

http://flybi.hms.harvard.edu/



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Contact

http://fgr.hms.harvard.edu/

flydiseasemodels.blogspot.com
flyrnai.blogspot.com

stephanie_mohr@hms.harvard.edu
@smohrfly