

in vivo CRISPR/Cas9 resources for gene overexpression and knockout

Jonathan Zirin, Harvard Medical School, Boston, MA

Why do we need a CRISPR library?

The "Phenotype Gap" – In FlyBase only 39% of *D. melanogaster* genes have molecular function information based on experimental evidence or inference from sequence or structural similarities.

Classical and insertion-based mutant alleles are available in stock centers for a similar fraction of sequence-located genes (6,146/14,898) and many of these have not been shown to alter gene function.

RNAi lines cover most of *Drosophila* protein-coding genes, but....

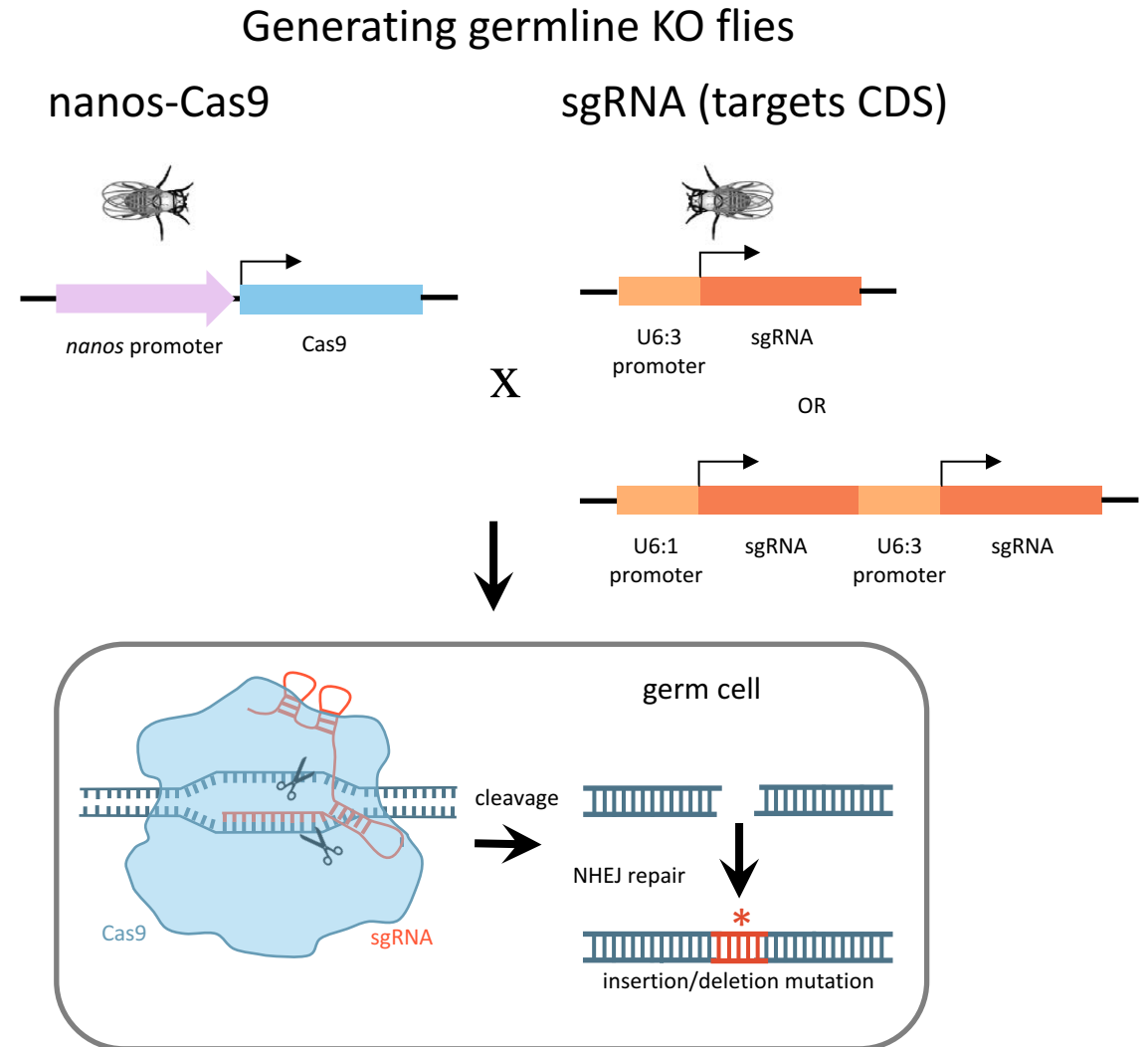
- Significant risks of false positive and false negative results
- Need 2+ RNAi lines per gene to confirm results

CRISPR/Cas9 can help address this Phenotype Gap

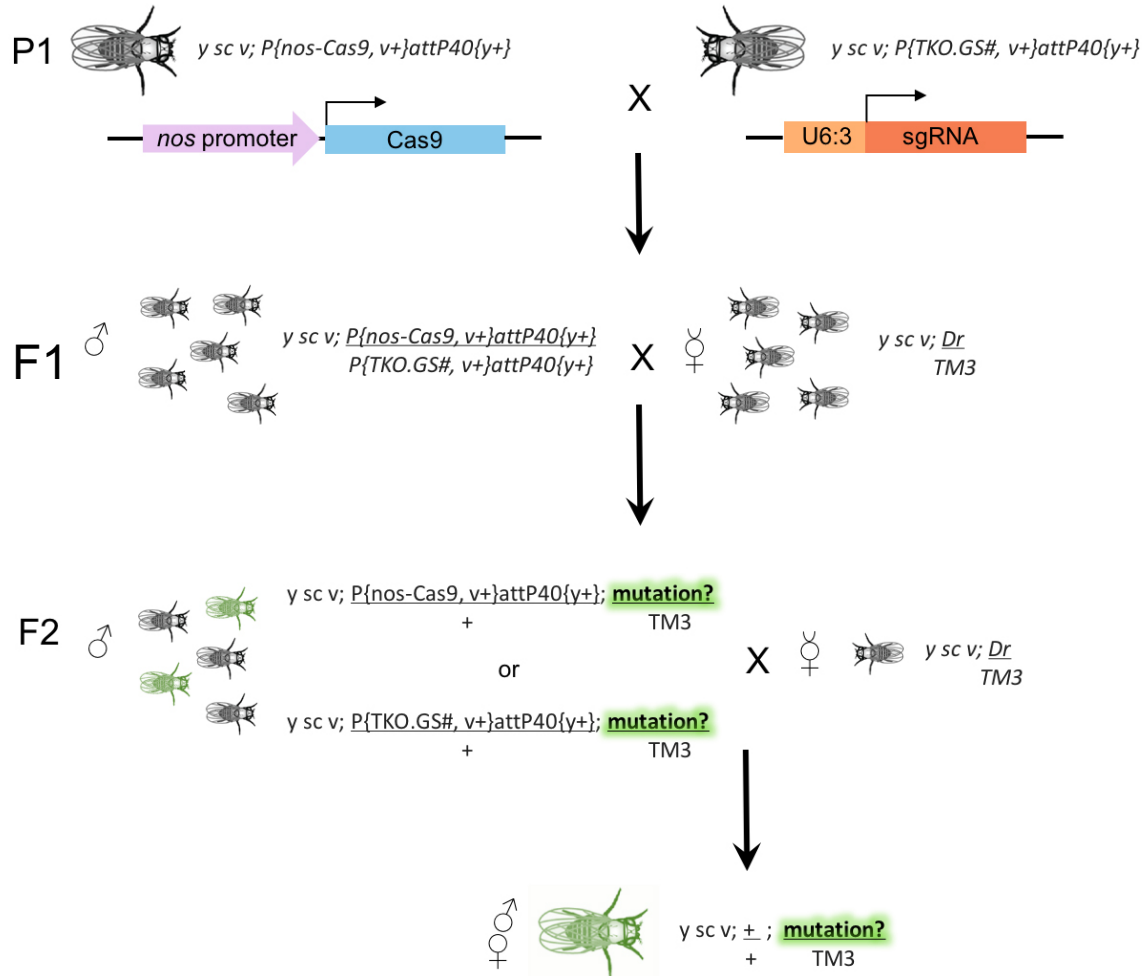
- Used in the germline to generate mutant stocks
- Used in the soma to generate mutant mosaics.
- dCas9 fusions for gene activation
- Genome engineering by homologous repair

TRiP-CRISPR Knockout (TRiP-KO)

- TRiP-KO flies ubiquitously express sgRNAs targeting gene coding sequence.
- Target most 5' sequence that will mutate all/most isoforms
- Mutant animals can be produced by simply crossing TRiP-KO flies to germline-specific-Cas9



Using TRiP-KO stocks to make mutants



Step 1: cross nanos-Cas9 (nos-Cas9) stock to individual TRiP-KO stock.

Step 2: collect at least 15 male F1 progeny containing both nos-Cas9 and sgRNA transgenes.

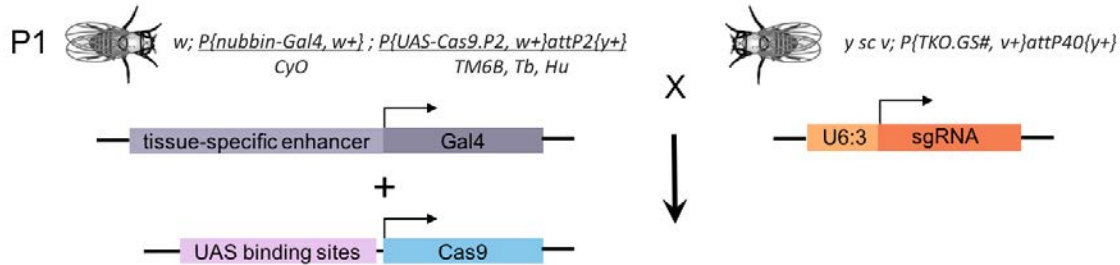
Step 3: cross male F1 progeny en masse to appropriate balancer strain for your target gene

Step 4: collect male or female F2 progeny (some will be heterozygous mutants) and cross each individually to balancer stock.

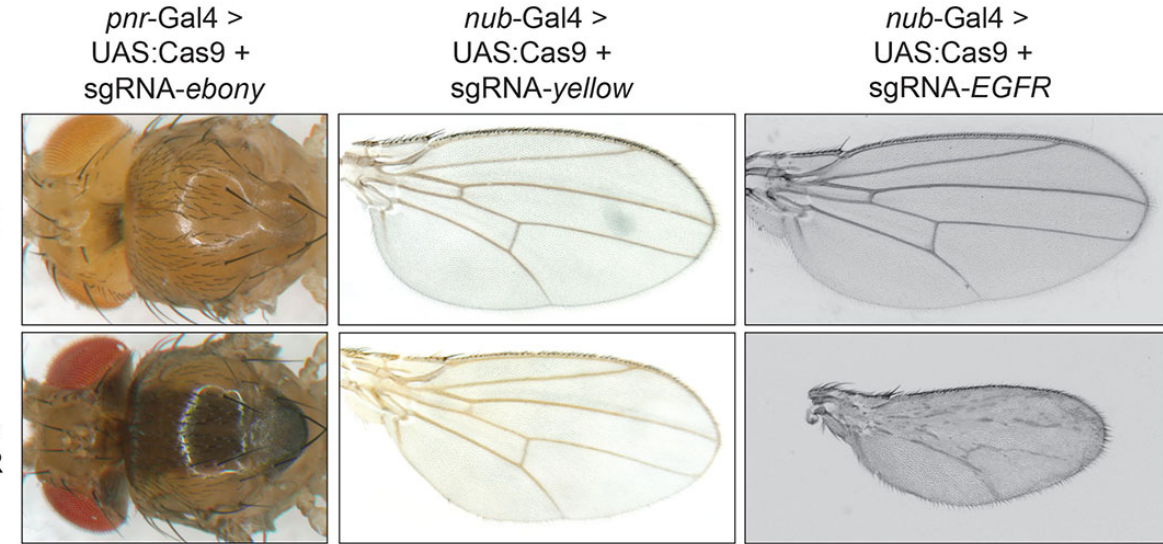
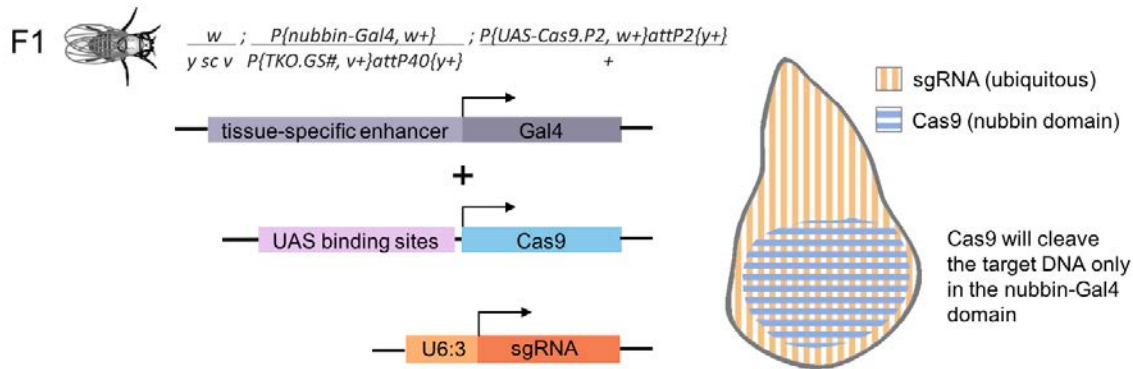
Step 5: Screen mutations by restriction profiling, endonuclease assays or high-resolution melt assays (HRMAs) and confirm by PCR and sequencing

Using TRiP-KO stocks for mosaic KO

Step 1: Step 1: cross tissue specific-Gal4 + UAS-Cas9 stock to individual TRiP-KO stock.



Step 2: collect male or female F1 progeny containing tissue-specific-Gal4, UAS-Cas9 and sgRNA transgenes and analyze phenotype.



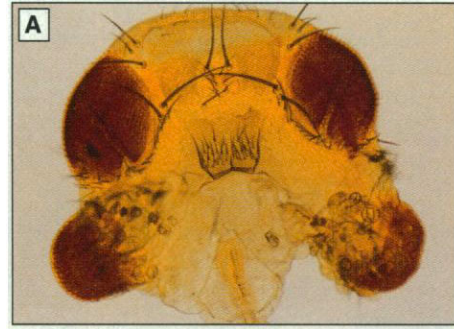
Two guides are better than one: the problem of in-frame deletions

CCATTGGCTCAGATTGACGAGGCGCCGGCAACTAAAAGA	WT
CCATTGGCTCAGATTGACG-----CCGGCAACTAAAAGA	*
CCATTGGCTCAGATTGA-----CGCCGGCAACTAAAAGA	*
CCATTGGCTCAGATTG-----GCCGGCAACTAAAAGA	*
CCATTGGCTCAGATTGACGAG-----CAACTAAAAGA	*
CCATTGGCTCAGATTGACGAa-----AACTAAAAGA	*
CCATTGGCTCAGA-----	*

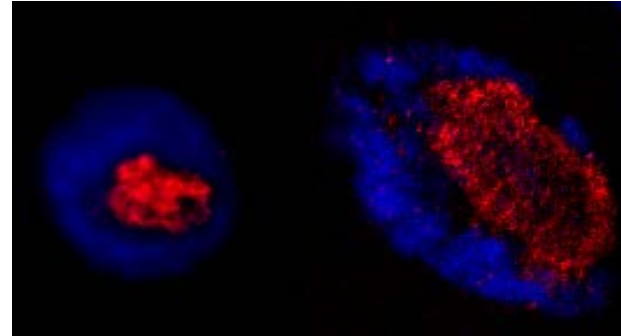
TRiP-CRISPR Overexpression (TRiP-OE)

Why overexpress a gene?

Overexpression of *eyeless* triggers a regulatory cascade that generates extra eyes



Eyeless overexpression
(Halder et al., 1995)



Myc overexpression
(Zirin and Perrimon, unpublished)

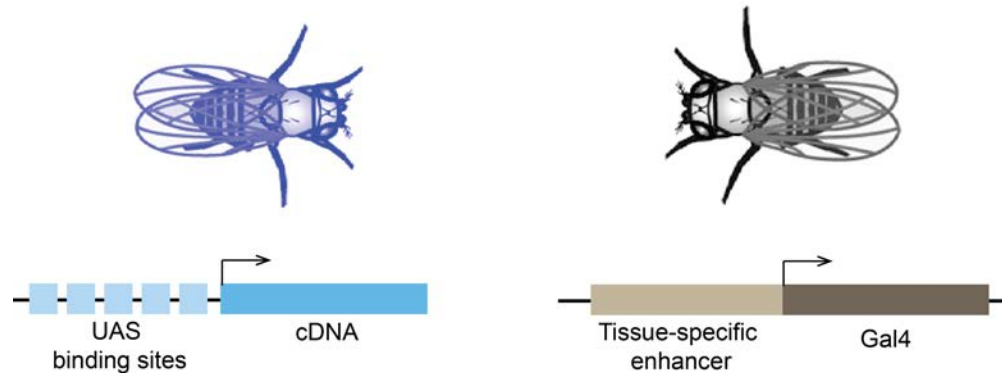
Overexpression of oncogenes the cause of many cancers. Here Myc overexpression causes the nucleolus to overgrow

Overexpression studies are useful for

- Determining the function of redundant genes (paralogs)
- Modifier screens
- Drug target screens

Current tools for over-expression in *Drosophila*

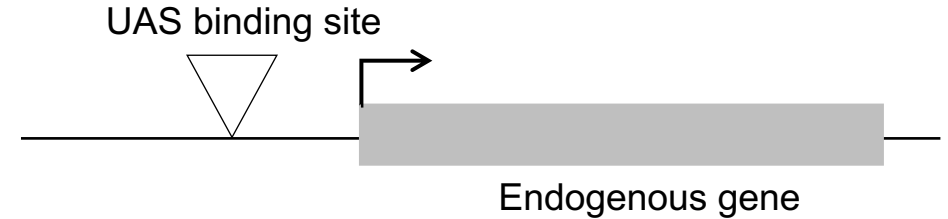
Gal4>UAS-cDNA



Limitations:

- 1) Expression is often at extremely high levels – possible toxicity
- 2) Cloning challenges – multiple isoforms, very large genes, etc.
- 3) Very hard to scale to a genome-wide resource

“EP Collection” (Rørth 1996)

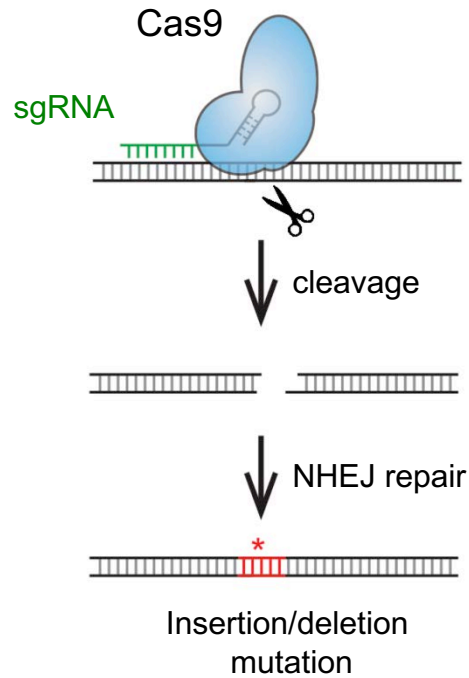


Limitations:

- 1) Expression is often very weak
- 2) Random integration
- 3) Disrupt gene

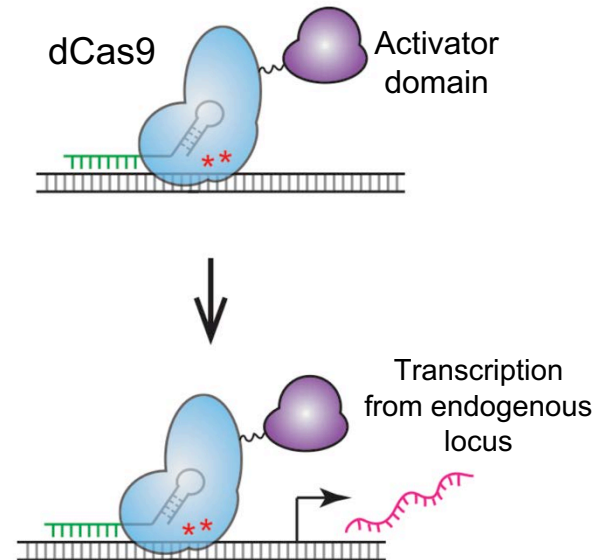
dCas9-fusions - a new tool for transcriptional activation

CRISPR



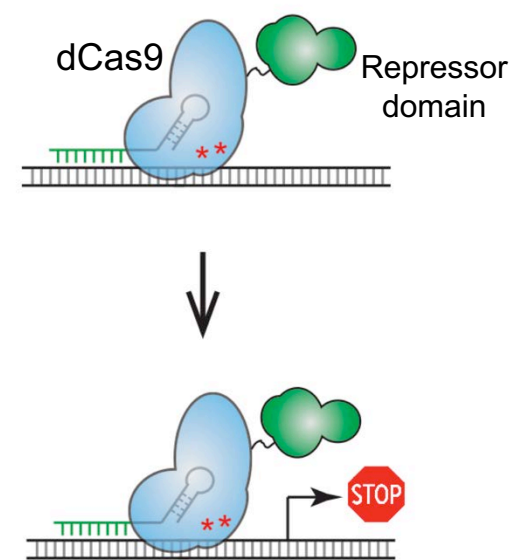
CRISPRa

Transcriptional activation



CRISPRi

Transcriptional repression



Point mutations D10A and H840A eliminate Cas9 endonuclease activity

VPR is a powerful Cas9 activator

Comparison of Cas9 activators in multiple species

Alejandro Chavez , Marcelle Tuttle, Benjamin W Pruitt, Ben Ewen-Campen, Raj Chari, Dmitry Ter-Ovanesyan, Sabina J Haque, Ryan J Cecchi, Emma J K Kowal, Joanna Buchthal, Benjamin E Housden, Norbert Perrimon, James J Collins & George Church 

Nature Methods **13**, 563–567 (2016)

doi:10.1038/nmeth.3871

Download Citation

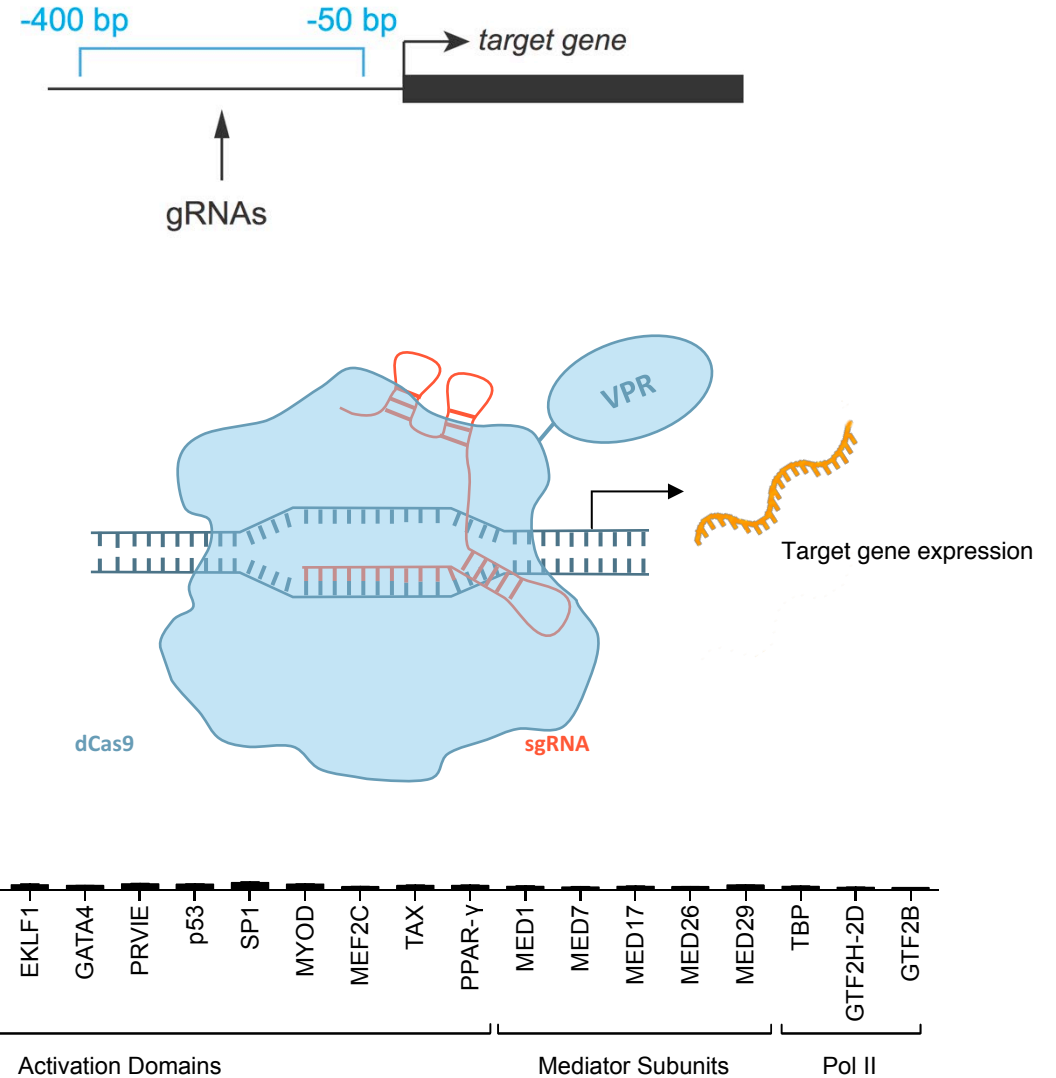
Genetic engineering Transcription

Received: 29 January 2016

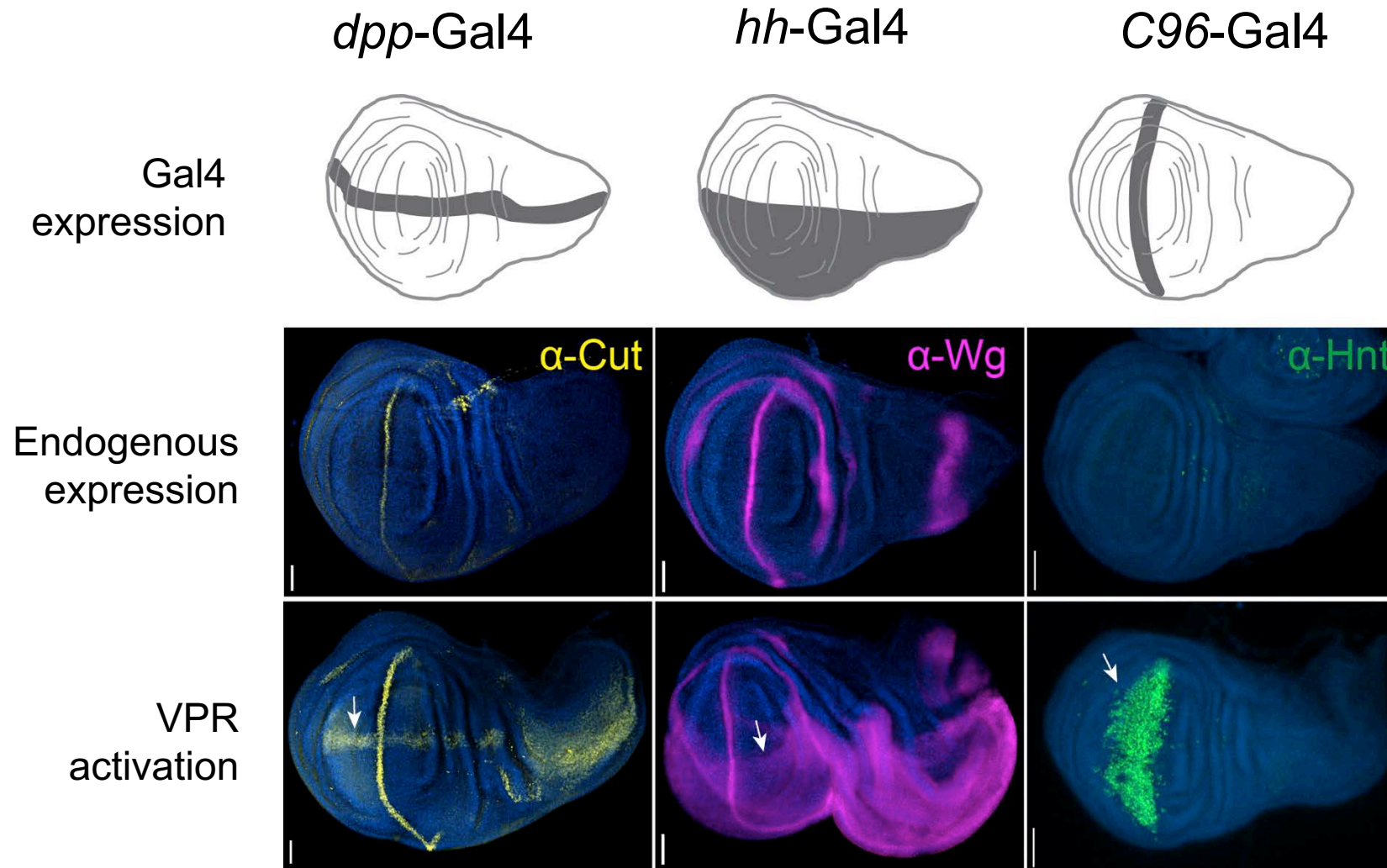
Accepted: 26 April 2016

Published: 23 May 2016

VP64-p65-Rta (VPR) – a tripartite activator, fused to nuclease-null Cas9

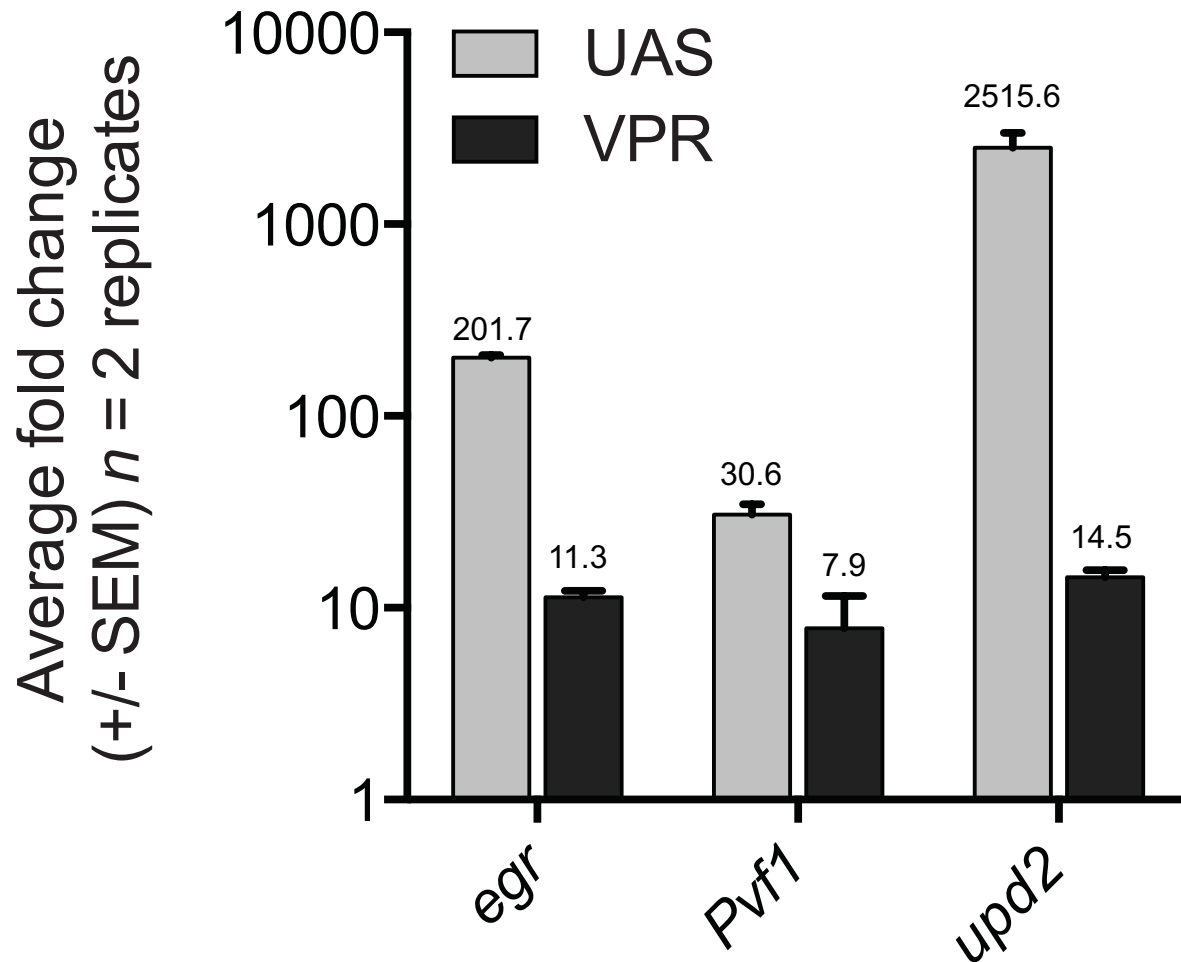


VPR activates a range of target genes *in vivo*



Images from
Ben Ewen-Campen

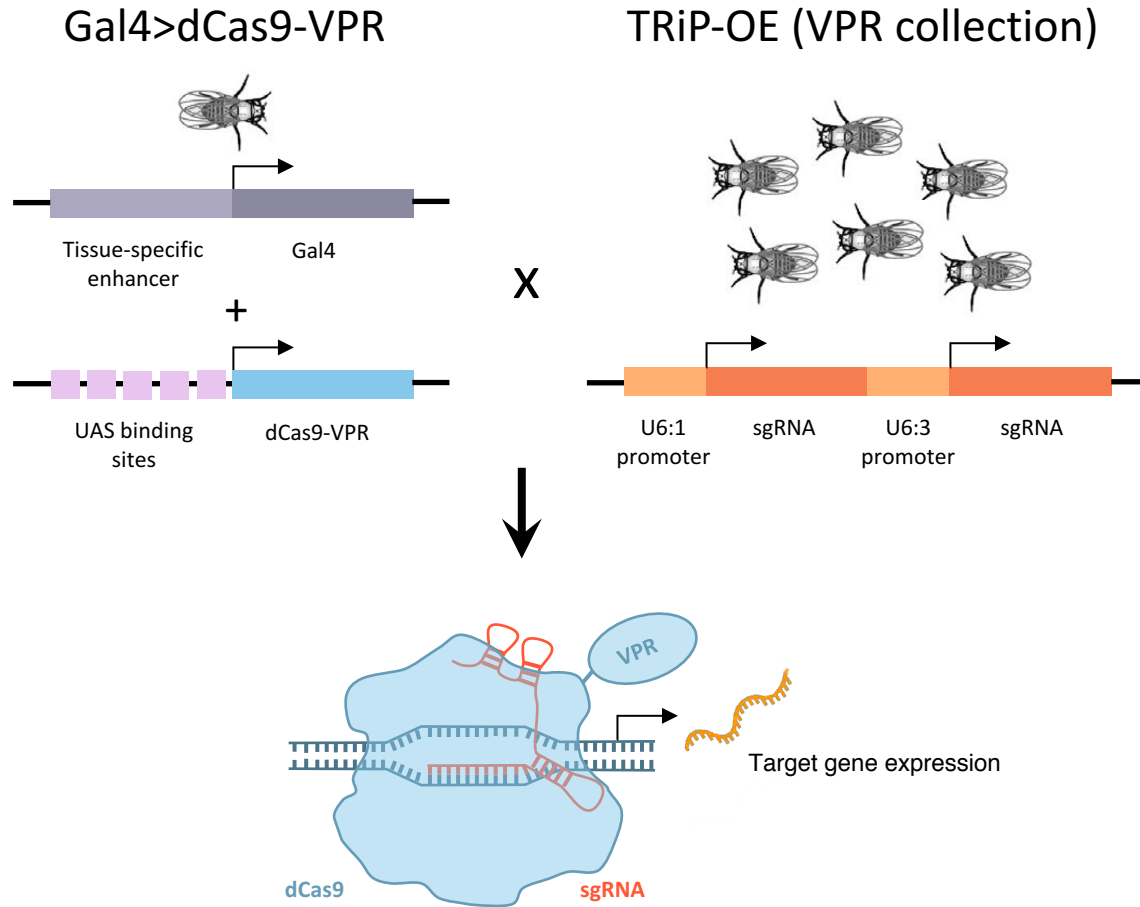
VPR vs UAS



- VPR is weaker than UAS
- Tested activation of 30 genes by VPR in vivo by qPCR
- from this group, almost 80% raised expression over 3-fold, and nearly 70% raised over 8-fold.

Data from Ben Ewen-Campen

The TRiP-OE VPR stock collection

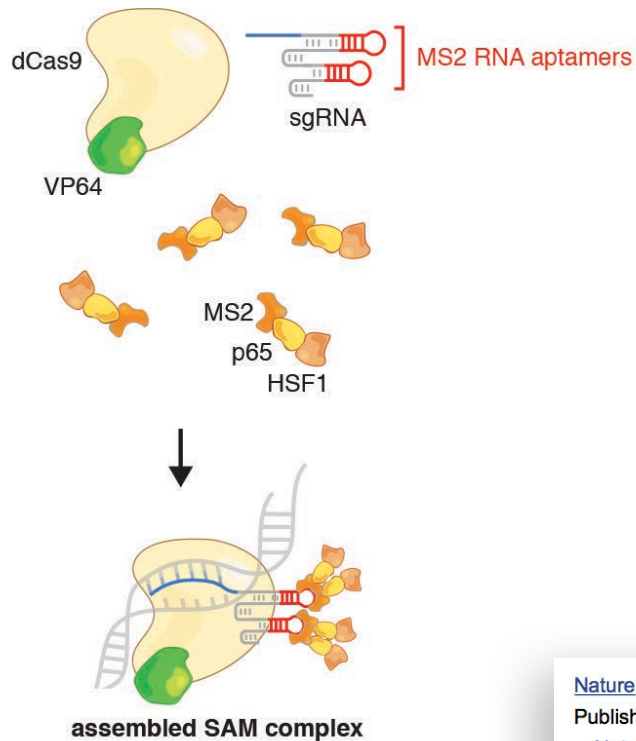


- A single gene is targeted by tandem expression of two sgRNAs from independent U6 promoters
- Stocks are made in the pCFD4 vector, developed by Phillip Port and colleagues
- Crossing TRiP-OE stocks to a Gal4 line expressing dCas9 fused to the chimeric activator domain VPR induces expression of the target gene
- Can also generate Indels and larger genomic deletions by crossing to Gal4>Cas9 flies
- Can only express wildtype proteins – no tags, no dominant negatives, etc..
- Can have off-targets if two genes are nearby.

Limitations of the VPR system

- 1) VPR typically requires two sgRNAs per target gene to reliably achieve consistent transcriptional activation, which greatly increases the cost and complexity of creating a large-scale resource for in vivo CRISPRa
- 2) Second, because CRISPRa requires three independent transgenes in a single fly (Gal4, UAS:dCas9-VPR, and sgRNA), the usage of this system is not as straightforward as standard Gal4-UAS based tools which only require a single genetic cross.
- 3) Experiments in *Drosophila* cell culture suggest that an alternative CRISPRa technique, synergistic activation mediator (SAM), outperforms VPR in direct comparisons. However, previous attempts to express SAM components in vivo have failed due to toxicity.

New OE library production based on SAM



CRISPR/Cas9 Synergistic Activation Mediator (SAM) is an engineered protein complex for the transcriptional activation of endogenous genes.

The SAM complex consists of three components:

- A nucleolytically inactive Cas9-VP64 fusion
- An sgRNA incorporating two MS2 RNA aptamers at the tetraloop and stem-loop
- The MCP-P65-HSF1 activation helper protein

The incorporation of three distinct activation domains - VP64, P65 and HSF1 – into the complex aids robust transcriptional activation through synergy.

[Nature](#). Author manuscript; available in PMC 2015 May 5.

Published in final edited form as:

[Nature](#). 2015 Jan 29; 517(7536): 583–588.

Published online 2014 Dec 10. doi: [10.1038/nature14136](https://doi.org/10.1038/nature14136)

PMCID: PMC4420636

NIHMSID: NIHMS681876

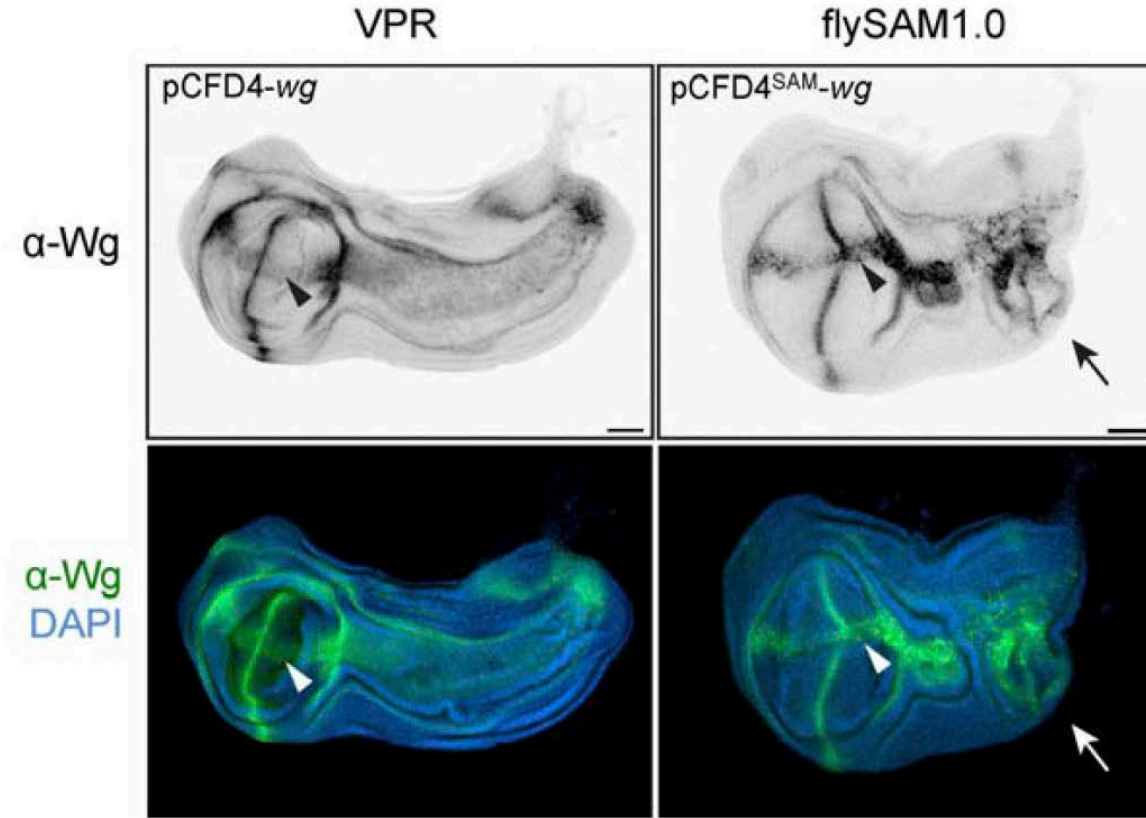
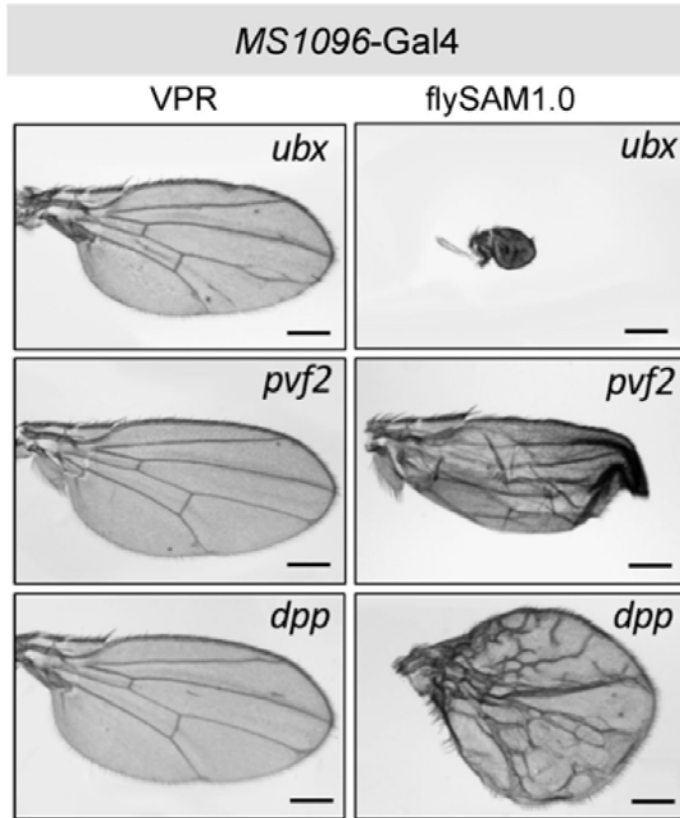
PMID: [25494202](https://pubmed.ncbi.nlm.nih.gov/25494202/)

Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex

[Silvana Konermann](#)^{1,2,3,4,*} [Mark D. Brigham](#)^{1,2,3,4,*} [Alexandro E. Trevino](#)^{1,2,3,4} [Julia Joung](#)^{1,4}
[Omar O. Abudayyeh](#)^{1,2,3,4} [Clea Barcena](#)^{1,2,3,4} [Patrick D. Hsu](#)^{1,2,3,4} [Naomi Habib](#)¹
[Jonathan S. Gootenberg](#)^{1,2,3,4,5} [Hiroshi Nishimasu](#)^{6,7} [Osamu Nureki](#)⁶ and [Feng Zhang](#)^{1,2,3,4,†}

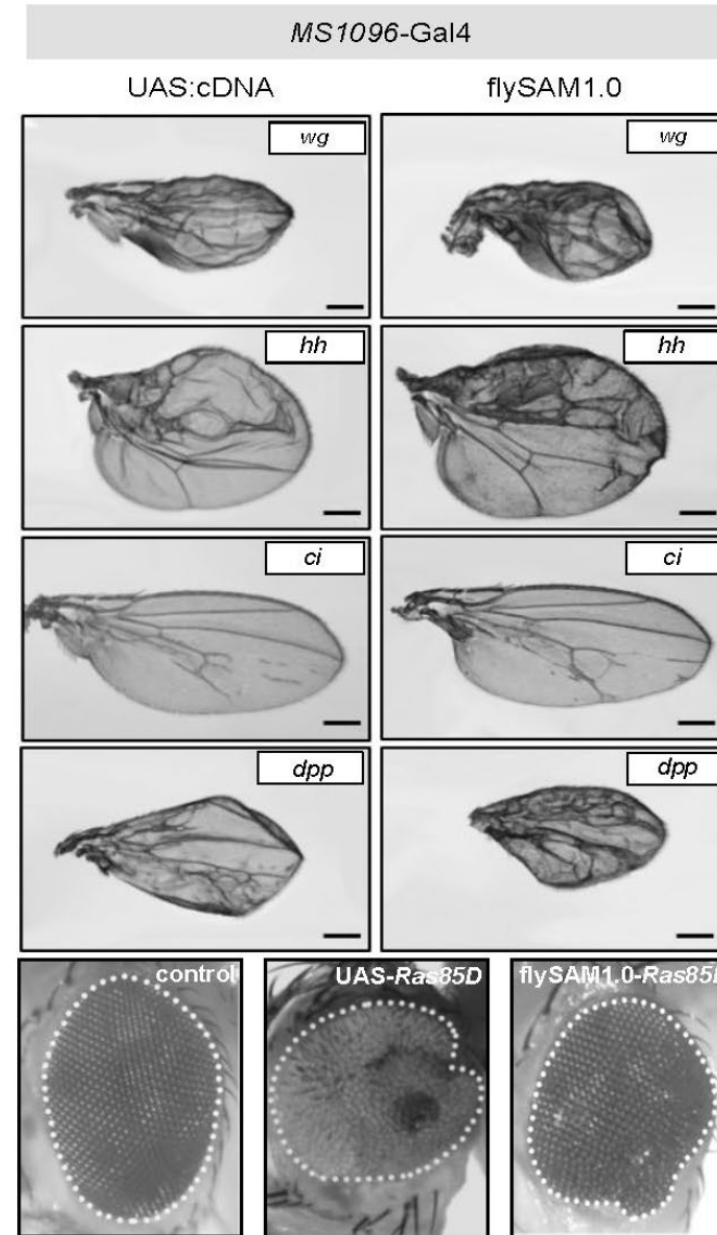
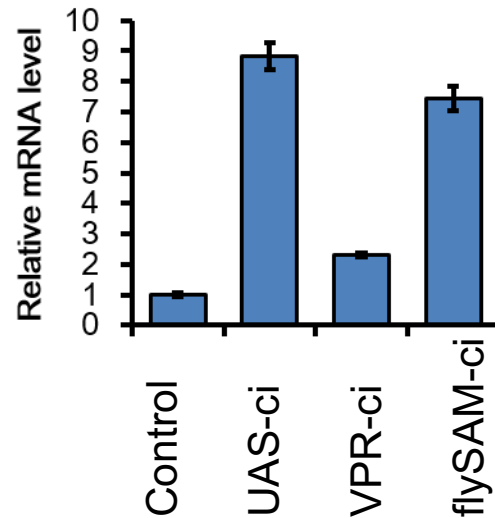
flySAM1.0

expresses the SAM components dCas9-VP64 and MCP-p65-HSF1 separated by a T2A self-cleaving peptide, under 10XUAS control

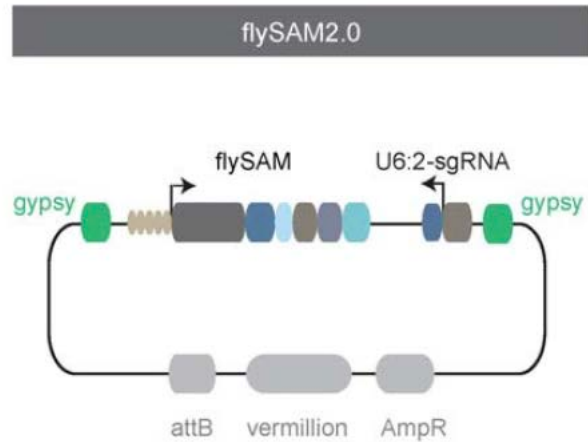


flySAM vs UAS

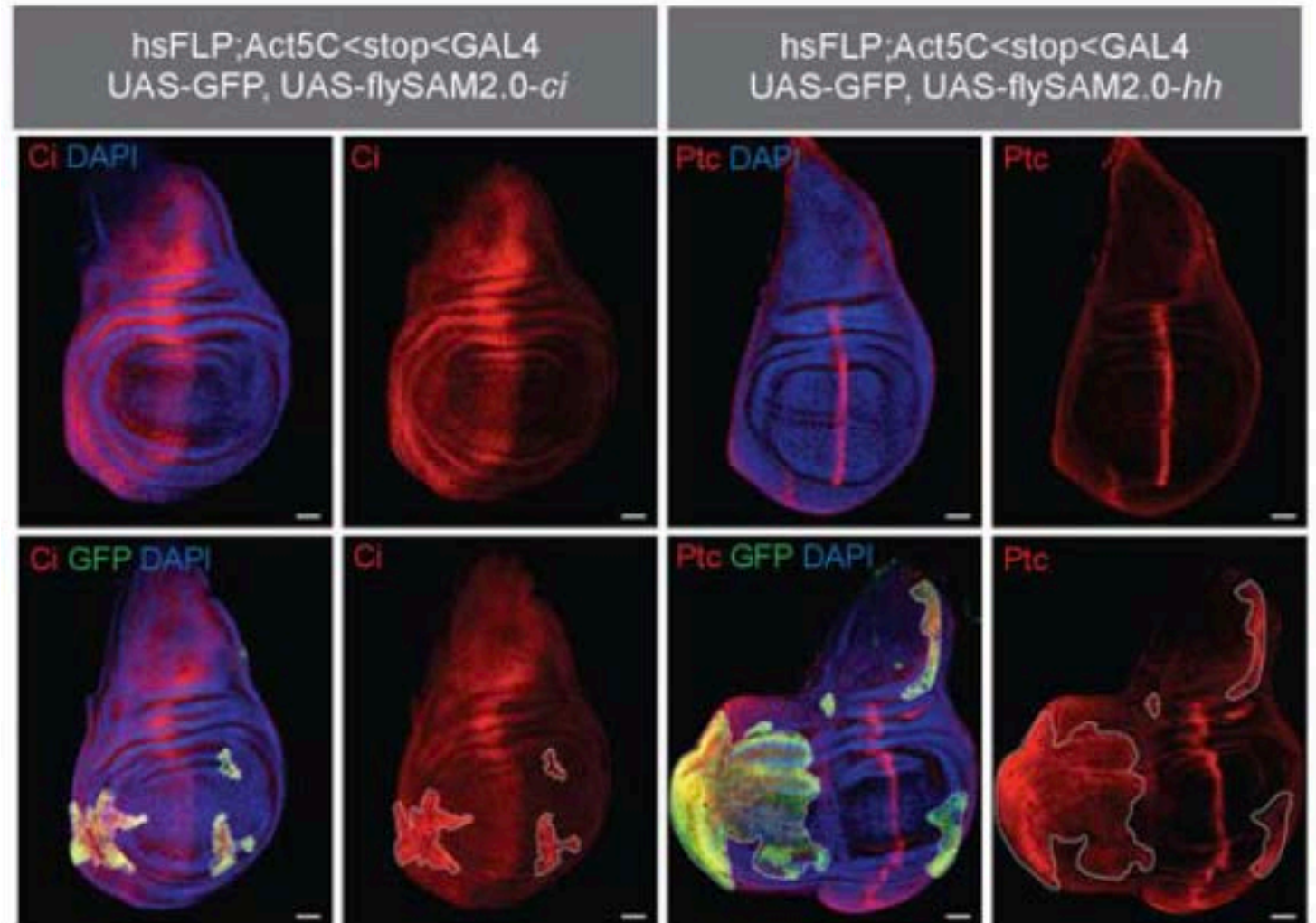
flySAM phenotypes recapitulate Gal4-UAS over-expression phenotypes.



flySAM2.0 contains both the UAS:flySAM and the sgRNA in a single plasmid



Clonal CRISPRa using
FLPout Gal4 > flySAM2.0
for *ci* and *hh* in L3 larval
wing discs



For more information
on CRISPRa in flies
check out these
publications

Optimized strategy for in vivo Cas9- activation in *Drosophila*

Ben Ewen-Campen, Donghui Yang-Zhou, Vitória R. Fernandes, Delfina P. González, Lu-Ping Liu, Rong Tao, Xingjie Ren, Jin Sun, Yanhui Hu, Jonathan Zirin, Stephanie E. Mohr, Jian-Quan Ni and Norbert Perrimon

PNAS August 29, 2017. 114 (35) 9409-9414; published ahead of print August 14, 2017.
<https://doi.org/10.1073/pnas.1707635114>

The screenshot shows the bioRxiv preprint server interface. At the top left is the Cold Spring Harbor Laboratory (CSH) logo. The bioRxiv logo is prominently displayed in the center, with the tagline 'THE PREPRINT SERVER FOR BIOLOGY'. In the top right corner, there are navigation links for 'HOME' and 'ABOUT', and a search bar. Below the header, the text 'New Results' is visible. The main title of the preprint is 'Next generation CRISPR/Cas9 transcriptional activation in *Drosophila* using flySAM'. The authors listed are Yu Jia, Rong-Gang Xu, Xingjie Ren, Ben Ewen-Campen, Rajendhran Rajakumar, Jonathan Zirin, Donghui Yang-Zhou, Ruibao Zhao, Fang Wang, Decai Mao, Ping Peng, Huan-Huan Qiao, Xia Wang, Lu-Ping Liu, Bowen Xu, Jun-Yuan Ji, Qingfei Lu, Jin Sun, Norbert Perrimon, and Jian-Quan Ni. The DOI is provided as <https://doi.org/10.1101/252031>. A note states: 'This article is a preprint and has not been peer-reviewed [what does this mean?].'. At the bottom of the preprint card, there are navigation options: 'Abstract' (highlighted with a purple bar), 'Info/History', 'Metrics', 'Supplementary material', and 'Preview PDF'.

In Vivo Transcriptional Activation Using CRISPR/Cas9 in *Drosophila*

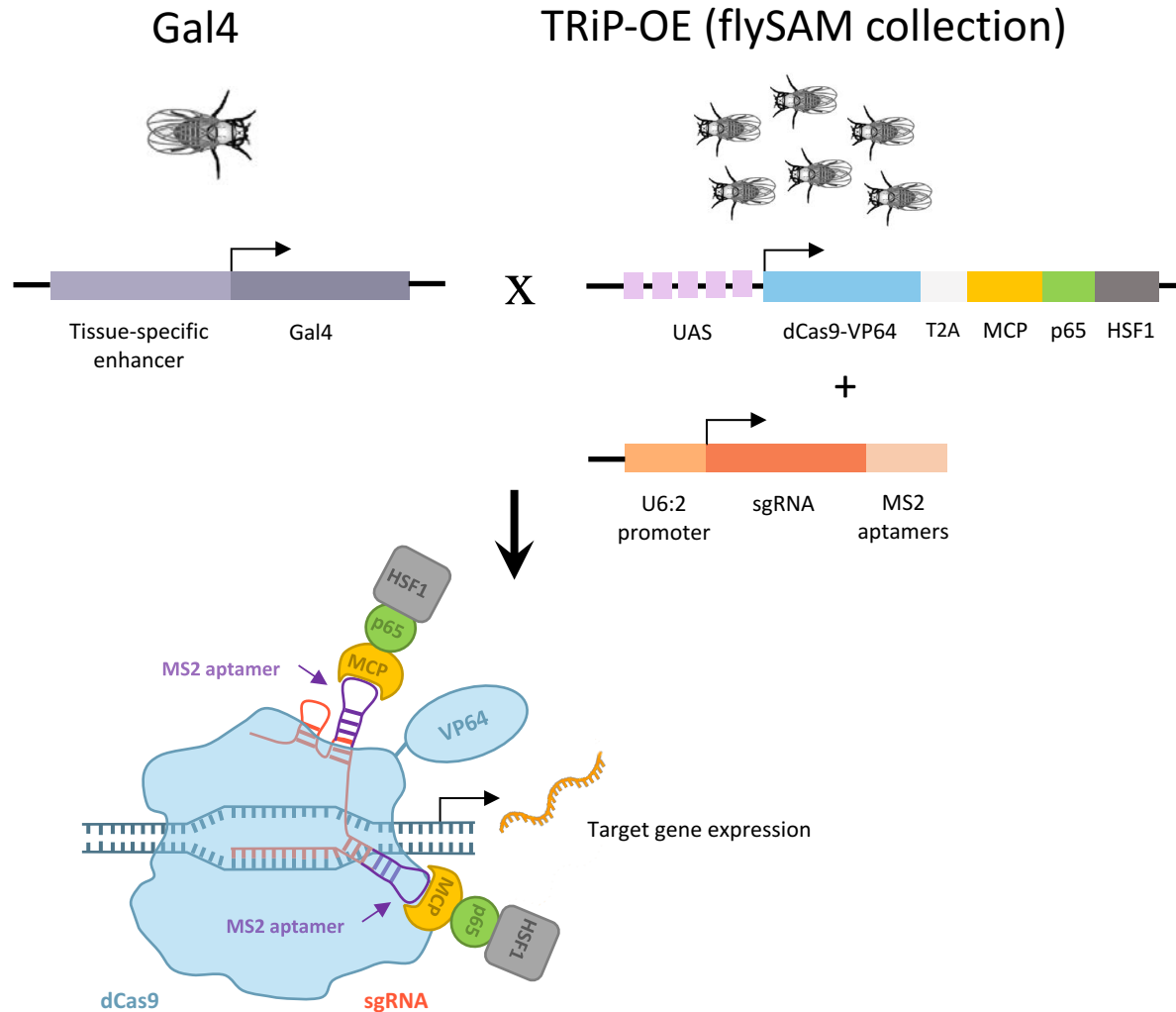
Shuailiang Lin, Ben Ewen-Campen, Xiaochun Ni,
Benjamin E. Housden and Norbert Perrimon

GENETICS October 1, 2015 vol. 201 no. 2 433-442;
<https://doi.org/10.1534/genetics.115.181065>

Article Figures & Data Supplemental Info & Metrics



The TRiP-OE flySAM stock collection



- New simplified strategy for *in vivo* CRISPR activation
- flySAM generates stronger overexpression phenotypes, comparable to Gal4/UAS
- A single gene is targeted by expression of one sgRNA from the U6:2 promoter
- Stocks are made in the flySAM2.0 vector, developed by Jian-quan Ni and colleagues
- TRiP-OE/flySAM stocks contain UAS-Cas9, so simply crossing to a Gal4 induces expression of the target gene

Search and nominate sgRNA lines

HARVARD MEDICAL SCHOOL

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DRSC/TRiP sgRNA Fly Stock Database

Search for **TRIP-CRISPR Overexpression (TRIP-OE)** and **TRIP-CRISPR Knockout (TRIP-KO)** fly stocks by gene or stock ID to obtain detailed information on sgRNA sequence, vector, and availability.

» Search stocks by:

Gene Identifiers (CG, FBgn, gene symbol)

GFP or GS number

nominate for in vivo CRISPR flies

nominate & track CRISPR-KO and -OE sgRNA fly stocks

Search

» N ← → luction

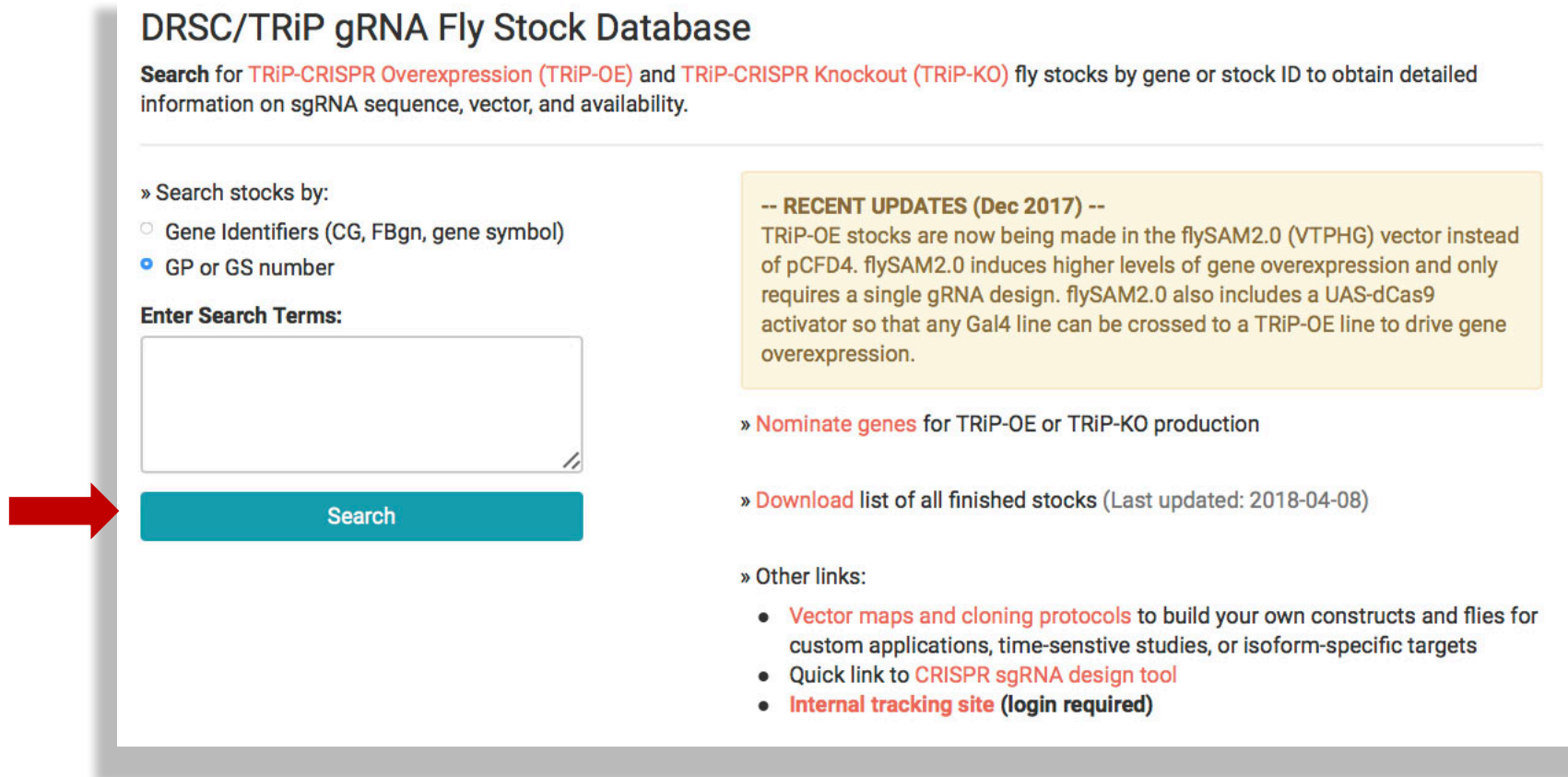
DRSC/TRiP Functional Genomics Resources

The DRSC/TRiP-FGR site joins the *Drosophila* RNAi Screening Center (DRSC) and Transgenic RNAi Project (TRiP). Check out our [Technologies](#), [Online Tools](#), and other pages to learn more.

Quick *direct* links to our most popular online search tools:

- [DIOPT ortholog search tool](#)
- [Gene Lookup](#) search of DRSC/TRiP reagents, data, etc.
- [UP-TORR batch search all public fly RNAi reagents](#)
- [RSVP search of in vivo fly RNAi data](#)
- [Find CRISPR gRNA search](#)
- [Search and Nominate](#) genes for TRiP-CRISPR fly stocks
- [Nominate genes for CRIMIC production](#)

sgRNA Fly Stock Database (http://www.flyrnai.org/tools/grna_tracker/)



DRSC/TRiP gRNA Fly Stock Database

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» Search stocks by:

- Gene Identifiers (CG, FBgn, gene symbol)
- GP or GS number

Enter Search Terms:

Search

-- RECENT UPDATES (Dec 2017) --
TRiP-OE stocks are now being made in the flySAM2.0 (VTPHG) vector instead of pCFD4. flySAM2.0 induces higher levels of gene overexpression and only requires a single gRNA design. flySAM2.0 also includes a UAS-dCas9 activator so that any Gal4 line can be crossed to a TRiP-OE line to drive gene overexpression.

» **Nominate genes** for TRiP-OE or TRiP-KO production

» **Download** list of all finished stocks (Last updated: 2018-04-08)

» Other links:

- **Vector maps and cloning protocols** to build your own constructs and flies for custom applications, time-sensitive studies, or isoform-specific targets
- Quick link to **CRISPR sgRNA design tool**
- **Internal tracking site** (login required)

Researchers can search TRiP-CRISPR stocks by gene identifier or by stock number and nominate genes for TRiP-OE or TRiP-KO production.

DRSC/TRiP gRNA Fly Stock Database

Search for **TRiP-CRISPR Overexpression (TRiP-OE)** and **TRiP-CRISPR Knockout (TRiP-KO)** fly stocks by gene or stock ID to obtain detailed information on sgRNA sequence, vector, and availability.

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- GP or GS number

Enter Search Terms:

Search

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[Export table](#)

Construct (GP ID)	Status	TRiP Stock (GS ID)	BDSC Stock ID	Function	Library	Type	Gene	Placement	Vector	Injection Site
GP00102	stock	GS00191	67560	Activation	TRiP-OE	Double sgRNA for 1 gene	hh	double sgRNA upstream of TSS	pCFD4	attP40
GP01881	stock	GS00602	76431	Cut (KO)	TRiP-KO	Single sgRNA	hh	CDS	pCFD3	attP40

Showing 1 to 2 of 2 entries

On the details page:

- General stock information
- sgRNA sequences
- Primer sequences

Request: GP00102

Request Data

Request_ID: 102
GP ID: GP00102
Construct Name Historical: pCFD4-hh
Scientist: Ben Ewen-Campen
Project: BEC36
sgRNA designed by: BEC
Initial Motivation: Activation
Library: TRIP-OE
Type: Double sgRNA for 1 gene
Experiment: Fly In vivo
Injection Site: attP40
Vector: pCFD4
Status: stock
Status Notes:
Comment:

sgRNA Data

sgRNA_ID: 687
sgRNA_seq: TATGCCACTCGACGTTGATCGG
sgRNA_seq_noPAM: TATGCCACTCGACGTTGAT
sgRNA_seq_noPAM_mod: GTATGCCACTCGACGTTGAT
sgRNA_seq_mod_rc: ATCGAACGTCGATGCGCATA
Target Gene: hh
Target CG: CG4637
Target FBgn: FBgn0004644
Placement: double sgRNA upstream of TSS
Note:

sgRNA_ID: 688
sgRNA_seq: TTCCACTTCCCTTGCGCATAAGG
sgRNA_seq_noPAM: TTCCACTTCCCTTGCGCATA
sgRNA_seq_noPAM_mod: GTTCCACTTCCCTTGCGCATA
sgRNA_seq_mod_rc: TATGCGCAAGGGAAGTGAAC
Target Gene: hh
Target CG: CG4637
Target FBgn: FBgn0004644
Placement: double sgRNA upstream of TSS
Note:

Primer Data

primer ID: 691
Primer Plate: IDT #9334043
Primer Well: H05
Primer Name: pCFD4-hh-F
Primer Type: forward
Primer Sequence: TATATAGGAAAGATATCCGGTGAACCTTCGTATGCCACTCGACGTTGATGTTTTAGAGCTAGAAATAGCAAG
primer ID: 692
Primer Plate: IDT #9334043
Primer Well: H06
Primer Name: pCFD4-hh-R
Primer Type: reverse
Primer Sequence: ATTTAACTTGCTATTCTAGCTCTAAACTATGCGCAAGGGAAGTGAACGACGTTAAATTGAAAATAGGTC

Stock Data

GP_ID: GP00102
Stock id: 191
Stock GS ID: GS00191
Stock Name Historical: SGR00122
Stock Status: finished
Stock Location: sgR05 : 02
Stock Date: 2015-11-04
Stock Note: finished before the implementation of tracking system
Injected Stock: y v nos-integrase ; attP40
Injection Date:
Injection Note: finished before the implementation of tracking system
Care Taker Larvae:
Obtain Transformant:
Genotype Balancer:
Genotype X Chr:
BDSC Stock ID: 67560
Genotype:

We welcome community nominations

DRSC/TRiP gRNA Fly Stock Database

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- Quick link to [CRISPR sgRNA design tool](#)
- [Internal tracking site](#) (login required)

Nominate Genes

» Step 1: Download the appropriate template

- If you only have the **gene information**[?], download this template: [\[icon\]](#)
- If you have both **gene and gRNA information**, download this template: [\[icon\]](#)
- If you have **gene, gRNA, and primer information**, download this template: [\[icon\]](#)

» Step 2: Enter the project information

🔔 TRIP-OE stocks are now being made in the flySAM2.0 (VTPHG) vector instead of pCFD4. flySAM2.0 induces higher levels of gene overexpression and only requires a single gRNA design. flySAM2.0 also includes a UAS-dCas9 activator so that any Gal4 line can be crossed to a TRIP-OE line to drive gene overexpression.

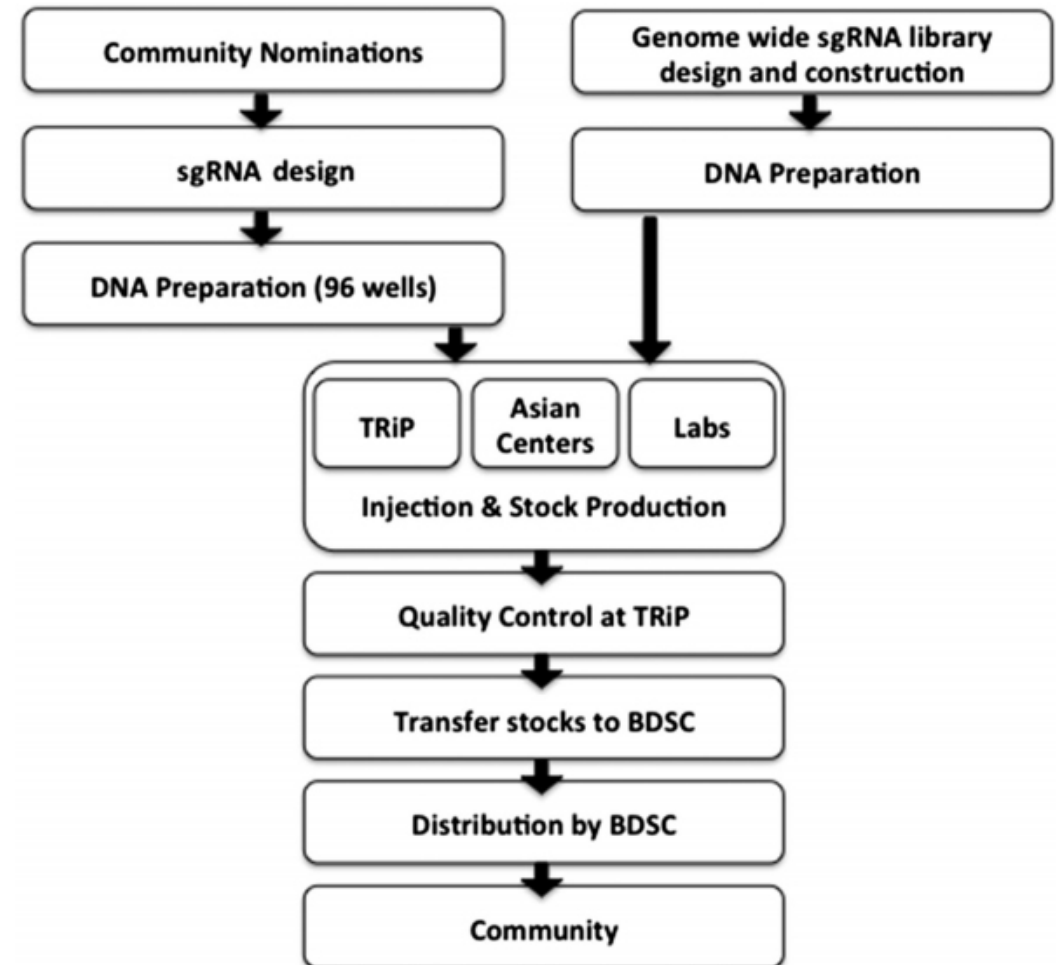
Scientist* <input type="text" value="Seth Brundle"/>	Function* <input type="text" value="Activation"/>
Project* <input type="text" value="cell transport"/>	Type* <input type="text" value="Single gRNA"/>
Email* <input type="text" value="brundlefly@bsl.edu"/>	Vector* <input type="text" value="flySAM2.0"/>
gRNA Designed By* <input type="text" value="Claire Hu"/> <small>Enter "Claire Hu" if you only have gene information – she will design the sgRNAs for you</small>	Target* <input type="text" value="Near TSS"/>
Comment <input type="text"/>	Experiment Type* <input type="text" value="Fly In vivo"/>
	Injection Site* <input type="text" value="attP40 (chr2)"/>

*required fields [Clear Experiment Details](#)

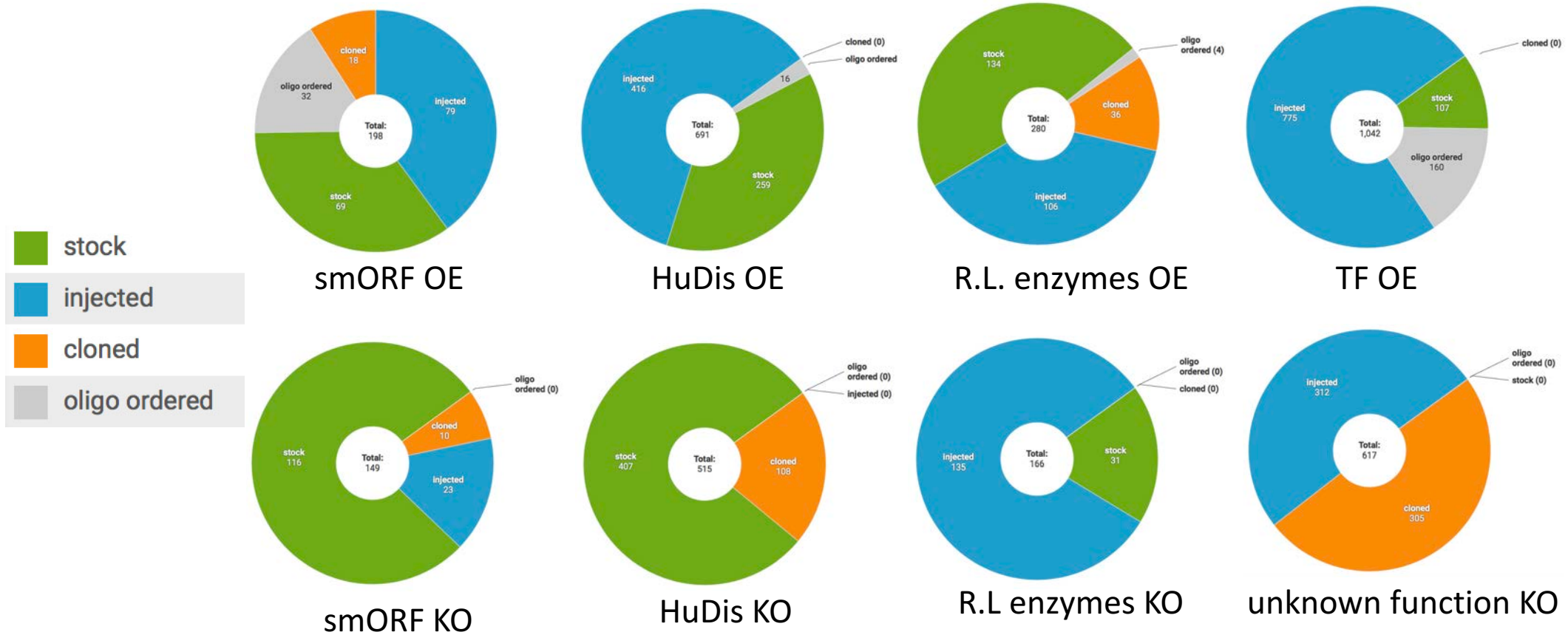
» Step 3: Upload the template file

No file chosen

Your nomination will immediately enter the TRiP production pipeline



TRiP-CRISPR production is focused on community nominations and mini-libraries



To date the TRiP has produced **~2000** sgRNA fly stocks for either gene overexpression or gene cutting, with **~1500** more constructs in the transformation pipeline.

TRiP-CRISPR Toolbox stocks

dCas9-activator stocks

- GAL4/UAS expression of Cas9 proteins with dead nuclease activity (dCas9), fused to VPR transcriptional activator (dCas9-VPR)
- GAL4/UAS combined with temperature-sensitive Gal80 (tubGal80[ts]) allows greater control of spatial and temporal dCas9-VPR expression

Stocks for mosaic knock-outs

GAL4/UAS expression of wild type Cas9


used for generating mutant mosaics in the soma in cells expressing sgRNAs targeting the coding region (eg. TRiP-KO stocks)

Stocks for germline mutants

germline-specific expression of wild type Cas9

used for generating small deletions and modifications in the germline in cells expressing sgRNAs targeting the coding region (eg. TRiP-KO stocks)

Search TRiP-CRISPR stocks at BDSC


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Genome Editing

- CRISPR - Cas9 and tracrRNA stocks
- CRISPR - Guide RNA Stocks**
- CRISPR - UAS-Cas9 & GAL4 (TRiP Toolbox set)
- I-SceI-directed Gene Targeting

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TRiP Toolbox Stocks for CRISPR Cas9

The Transgenic RNAi Project (TRiP) is producing guide RNA transgenes for both overexpression and knockout of genes via CRISPR/Cas9 mechanisms. The stocks listed below were made by TRiP to facilitate use of these stocks.

P{UAS-3xFLAG.dCas9.VPR} expresses a nuclease-dead Cas9 fused to a chimeric activation domain under the control of UAS. It can be used to activate transcription of a gene in the presence of a guide RNA designed to facilitate overexpression of that gene. For more details see the TRiP-CRISPR overexpression site. The stocks below pair UAS-Cas9.VPR with various GAL4 and/or GAL80 insertions.

UAS-Cas9.VPR stocks

Cas9	GAL4	GAL80	Stock Number	Genotype
P{UAS-3xFLAG.dCas9.VPR}attP2	P{bs-GAL4.Term}G1		67051	w[*]; P{w+mC}=bs-GAL4.Term}G1/CyO; P{w+mC}=UAS-3xFLAG.dCas9.VPR}attP2
P{UAS-3xFLAG.dCas9.VPR}attP2	P{bs-GAL4.Term}G1	P{tubP-GAL80[ts]}2	67071	w[*]; P{w+mC}=bs-GAL4.Term}G1/CyO; P{w+mC}=UAS-3xFLAG.dCas9.VPR}attP2; P{w+mC}=tubP-GAL80[ts]}2
P{UAS-3xFLAG.dCas9.VPR}attP2	P{en2.4-GAL4}e16E		67049	w[*]; P{w+mW.hs]=en2.4-GAL4}e16E; P{w+mC}=UAS-2xEGFP}AH2/CyO; P{w+mC}=UAS-3xFLAG.dCas9.VPR}attP2
P{UAS-3xFLAG.dCas9.VPR}attP2	P{en2.4-GAL4}e16E	P{tubP-GAL80[ts]}2	67069	w[*]; P{w+mW.hs]=en2.4-GAL4}e16E; P{w+mC}=UAS-2xEGFP}AH2/CyO; P{w+mC}=UAS-3xFLAG.dCas9.VPR}attP2; P{w+mC}=tubP-GAL80[ts]}2
P{UAS-3xFLAG.dCas9.VPR}attP2	P{GAL4-nos.NGT}40		67052	w[*]; P{w+mC}=GAL4-nos.NGT}40/CyO; P{w+mC}=UAS-3xFLAG.dCas9.VPR}attP2
P{UAS-3xFLAG.dCas9.VPR}attP2	P{GAL4-twi.2xPE}1		67053	w[*]; P{w+mC}=GAL4-twi.2xPE}1/CyO; P{w+mC}=UAS-3xFLAG.dCas9.VPR}attP2

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The TRiP encourages the community to nominate genes for TRiP-OE and TRiP-KO production via the TRiP sgRNA Database. If use of any of the TRiP-CRISPR stocks results in data used in a publication, please cite the TRiP in your publication.

** Some guide RNAs target more than one gene. In the tables below, the "# Targets" column indicates the number of targets per transgene. To see the other targets, either filter the table with the stock number or click on the stock number to read the stock report.

Control stock

Stk #	Assoc. Gene	# Targets**	Vector	Symbol	Chr
67539	QUAS	1	pCFD4	P{GS00089}attP40	2

TRiP-CRISPR Overexpression (TRiP-OE) stocks

Stk #	Assoc. Gene	# Targets**	Vector	Symbol	Chr
68079	Acon	1	pCFD4	P{TOE.GS00476}attP40	2
68080	Acox57D-d	1	pCFD4	P{TOE.GS00477}attP40	2
68081	Acox57D-p	1	pCFD4	P{TOE.GS00478}attP40	2
67641	Actbeta	1	pCFD4	P{TOE.GS00028}attP40	2
76109	AdenoK	1	pCFD4	P{TOE.GS00750}attP40	2
68082	Ady43A	1	pCFD4	P{TOE.GS00479}attP40	2
77226	AlaRS	1	pCFD4	P{TOE.GS01039}attP40	2
68083	Alas	1	pCFD4	P{TOE.GS00480}attP40	2
68084	Aldh	1	pCFD4	P{TOE.GS00481}attP40	2
67602	alpha-Man-Ib	2	pCFD4	P{TOE.GS00397}attP40	2
67531	aos	1	pCFD4	P{TOE.GS00057}attP40	2

Search and add feedback for sgRNA lines at RSVP *Plus*

http://www.flyrnai.org/cgi-bin/RSVP_search.pl

RSVP *Plus*
RNAi Stock Validation & Phenotypes
Harvard Medical School

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Search by term(s), driver(s), or a combination:

ct|

(gene symbol, CG, FBgn, or TRiP line)

Driver(s)

Any Driver
△15-8,477
0104
1003.3
109(2)80
109-79
11-81

Only show lines with RSVP data.

Search gene symbol, CG, FBgn, Stock ID (TRiP, VDRC or NIG)
 Search BDSC Stock ID

Search

RNAi Stock Validation and Phenotype *Plus* (RSVP *Plus*) allows for search and view of RNAi **and sgRNA** fly stocks from TRiP, VDRC, and NIG-Japan, along with any available data regarding knockdown or phenotypes observed for that fly stock and a given Gal4 driver (e.g. from publications).

RSVP *Plus*

RNAi Stock Validation & Phenotypes

Harvard Medical School

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Search by term(s), driver(s), or a combination:

ct

(gene symbol, CG, FBgn, or TRiP line)

Driver(s)

Any Driver
 △15-8,477
 0104
 1003.3
 109(2)80
 109-79
 11-81

Only show lines with RSVP data.

- Search gene symbol, CG, FBgn, Stock ID (TRiP, VDRC or NIG)
- Search BDSC Stock ID

Search

Download the Results (.xls format, with data summary).

	FBgn	CG	Gene Name	BDSC Stock ID	Detail Page	Line Source	Vector	Stock Type	Insert Site	Data
1	FBgn0004198	CG11387	cut	-	5687	VDRG-GD		RNAi		pnr-MD237
2	FBgn0004198	CG11387	cut	67524	GS00041	TRiP	pCFD4	sgRNA-OE	attP40	P{w[+mW.hs]=GAL4-dpp.blk1}
3	FBgn0004198	CG11387	cut	33967	HMS00924	TRiP	VALIUM20	RNAi	attP2	Act5C-Gal4 ppk.1.9 MTD-Gal4
4	FBgn0004198	CG11387	cut	29625	JF03304	TRiP	VALIUM10	RNAi	attP2	Act5C-Gal4 ppk.1.9 y w; UAS-dcr2; nanos-Gal4

4 results (1 genes).

Download the Results (.xls format, with data summary).



P{TRIP.GS00041}attP40

Gene Information

Symbol: ct
CG: CG11387
FBgn: FBgn0004198

TRIP Stock Information

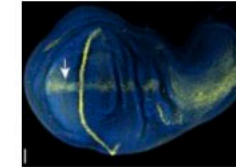
Collection: TRIP **Line ID:** [GS00041](#) **Type:** sgRNA-OE
BDSC ID: [67524](#)
Vector: [pCFD4](#) **Insertion Site:** attP40

Validation Test Results

Driver: P{w[+mW.hs]=GAL4-dpp.blk1}
Driver BDSC: [67045](#)
Contributor(s): Jonathan Zirin
Experiment Type : Activation
Reference: [Optimized strategy for in vivo Cas9-activation in Drosophila.](#) [PUBMED](#) [PDF](#)

Phenotype Results:

- **Phenotype:** ectopic cut expression: a stripe of ectopic Cut expression along the A/P boundary of the wing disc
- **Phenotype Anitbody Info:** mouse anti-Cut (2B10, DSHB, 1:10)
- **Phenotype Category:** wing imaginal disc
- **Temperature (°C):** 27.0



Community Input (asterisk indicates required field)

Contributor* :	<input type="text"/>	Describe the phenotype.
Driver* :	<input type="text"/>	
Driver BDSC:	<input type="text"/>	
Temperature* :	<input type="text"/> °C	
qPCR data:	<input type="text"/> % Remaining after knockdown	
Cas9 variant* :	<input type="text" value="Cas9.P2 (cutting)"/>	Other: <input type="text"/>
experiment:	<input type="text" value="knock-out"/>	Other: <input type="text"/>
Phenotype?	<input type="radio"/> As expected <input type="radio"/> None detected <input type="radio"/> Unclear (difficult to determine phenotype) <input type="radio"/> Novel (unexpected phenotype)	
Contributor Email* :	<input type="text" value="For follow-up only."/>	

Submit Input

P{TRIP.GS00041}attP40

Gene Information

Symbol: ct
CG: CG11387
FBgn: FBgn0004198

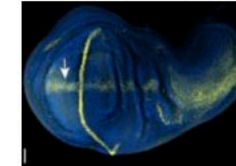
TRIP Stock Information

Collection: TRIP **Line ID:** [GS00041](#) **Type:** sgRNA-OE
BDSC ID: [67524](#)
Vector: [pCFD4](#) **Insertion Site:** attP40

Validation Test Results

Driver: P{w[+mW.hs]=GAL4-dpp.blk1}
Driver BDSC: [67045](#)
Contributor(s): Jonathan Zirin
Experiment Type: Activation
Reference: [Optimized strategy for in vivo Cas9-activation in Drosophila](#). [PUBMED](#) [PDF](#)

- Phenotype Results:**
- **Phenotype:** ectopic cut expression: a stripe of ectopic Cut expression along the A/P boundary of the wing disc
 - **Phenotype Antibody Info:** mouse anti-Cut (2B10, DSHB, 1:10)
 - **Phenotype Category:** wing imaginal disc
 - **Temperature (°C):** 27.0



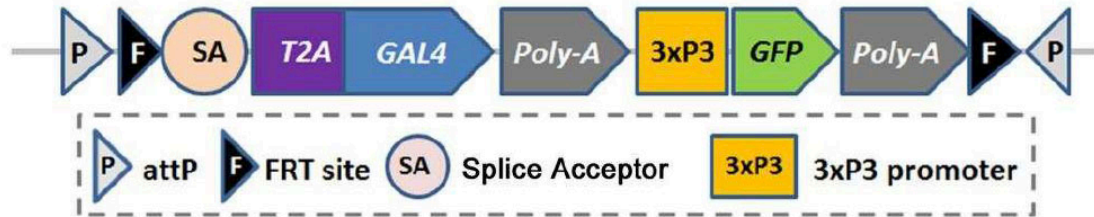
Community Input (asterisk indicates required field)

Contributor*:	<input type="text"/>	Describe the phenotype.
Driver*:	<input type="text"/>	
Driver BDSC:	<input type="text"/>	
Temperature*:	<input type="text"/> °C	
qPCR data:	<input type="text"/> % Remaining after knockdown	
Cas9 variant*:	<input checked="" type="checkbox"/> Cas9.P2 (cutting) <input type="checkbox"/> Cas9.P (cutting) <input type="checkbox"/> dCas9.VPR (activation) <input type="checkbox"/> dCas9.VP64 (activation, flySAM) <input type="checkbox"/> other (please specify)	Other: <input type="text"/>
experiment:		Other: <input type="text"/>
Phenotype?		<input type="checkbox"/> r (difficult to determine phenotype) <input type="checkbox"/> Novel (unexpected phenotype)
Contributor		<input type="text"/>
Email*:		<input type="text"/>

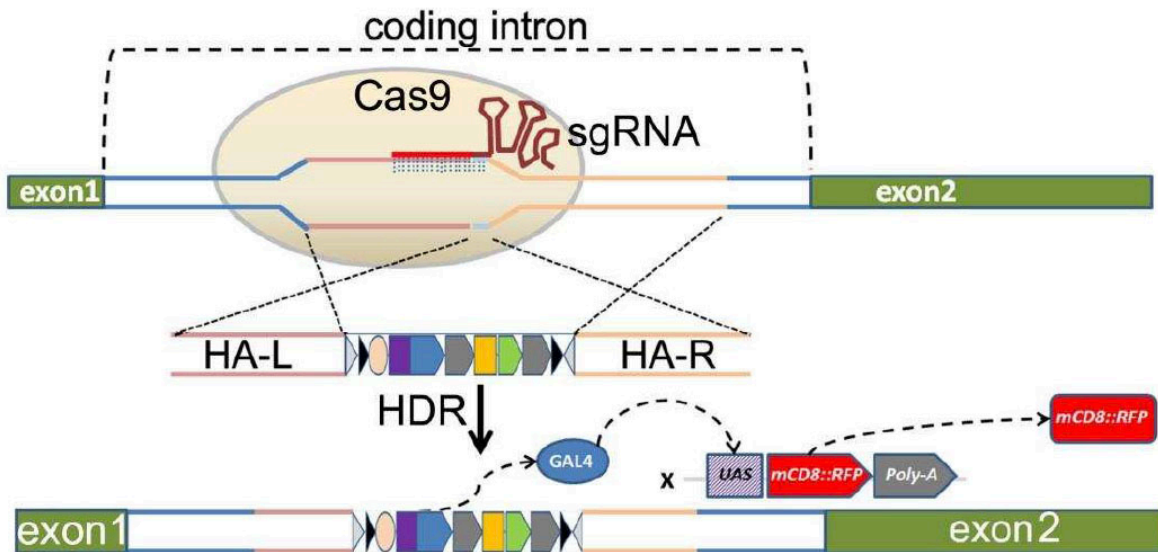
Submit Input

The CRIMIC Project

Structure of CRIMIC (CRISPR Mediated Integration Cassette) cassette (pM37)



Schematic of pM37 integration by CRISPR/Cas9



Collaboration with Hugo Bellen's group

Insert gene traps into the 5'-most intron that can be used to tag all or most of the predicted splice Isoforms.

A gene-specific *T2A-GAL4* library for *Drosophila*



Pei-Tseng Lee, Jonathan Zirin, Oguz Kanca, Wen-Wen Lin, Karen L Schulze, David Li-Kroeger, Rong Tao, Colby Devereaux, Yanhui Hu [see all](#)
 Baylor College of Medicine, United States; Harvard Medical School, United States; Howard Hughes Medical Institute, Carnegie Institution for Science, United States

Nominate your gene for CRIMIC

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DRSC/TRiP Functional Genomics Resources

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DRSC/TRiP sgRNA Fly Stock Database

Search for **TRIP-CRISPR Overexpression (TRiP-OE)** and **TRIP-CRISPR Knockout (TRiP-KO)** fly stocks by gene or stock ID to obtain detailed information on sgRNA sequence, vector, and availability.

Search stocks by:

- Gene Identifiers (CG, FBgn, gene symbol)
- DB ID number

n nominate for in vivo CRISPR flies
nominate & track CRISPR-KO and -OE sgRNA fly stocks


Search

DRSC/TRiP Functional Genomics Resources

The DRSC/TRiP-FGR site joins the *Drosophila* RNAi Screening Center (DRSC) and Transgenic RNAi Project (TRiP). Check out our [Technologies](#), [Online Tools](#), and other pages to learn more.

Quick *direct* links to our most popular online search tools:

- [DIOPT ortholog search tool](#)
- [Gene Lookup search of DRSC/TRiP reagents, data, etc.](#)
- [UP-TORR batch search all public fly RNAi reagents](#)
- [RSVP search of in vivo fly RNAi data](#)
- [Find CRISPR gRNA search](#)
- [Search and Nominate genes for TRiP-CRISPR fly stocks](#)
- [Nominate genes for CRIMIC production](#)



<http://www.flyrnai.org/tools/crimic/web/>

CRIMIC CRISPR MiMIC Gene Trap Method

For genes that permit intronic tagging, we design homology arms within the 5'-most intron that can be used to tag all or most of the predicted splice isoforms. These homology arms are cloned into a donor vector such that they flank a mutagenic CRISPR MiMIC (CRIMIC) gene trap, containing a splice acceptor, stop codons, as well as a T2A-Gal4. Injection of the construct and an sgRNA targeting the intron into Cas9-expressing fly embryos induces a DNA double-strand break in the germline, which allows for integration of the CRIMIC trap by homology directed repair. Once integrated, the CRIMIC insert produces a truncated mRNA of the target gene, as well as Gal4 under the control of the endogenous gene regulatory elements. These cassettes can then be converted into protein traps using established RMCE methods.

Check Status Use the search box below to check the nomination status of a gene. You may enter a FBgn, gene symbol, or CG.

Note: some genes may have more than one nomination.

Nominate Gene List Before submitting your nomination, please check the **MiMIC database** to verify if your gene does not already have a T2A-Gal4 line or a MiMIC insertion suitable for Recombination Mediated Cassette Exchange (RMCE).

Scientist*

Email*

PI*

Institute*

Project Name*

Gene List*

Use this template to upload your gene list: [\[XLS\]](#)

We ask that you try to limit your nomination to 10 genes or less. It can be more, but the project manager may contact you for more details.

Choose File no file selected

Note: FBgn is required but gene annotation (CG) and symbol can be left blank. Any rows with missing FBgns will not be nominated.

Comment

*required fields

Submit

Clear

Acknowledgements

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Howard Hughes Medical Institute

PgmNr 867: Cell-based screen technologies at the Drosophila RNAi Screening Center.

PgmNr 869: A new resource from the TRiP: sgRNA stocks for gene overexpression and knockout by CRISPR-Cas9.

PgmNr 876: DRSC Informatics Tools for Functional Genomics Studies – 2018 update.