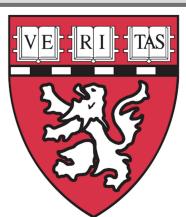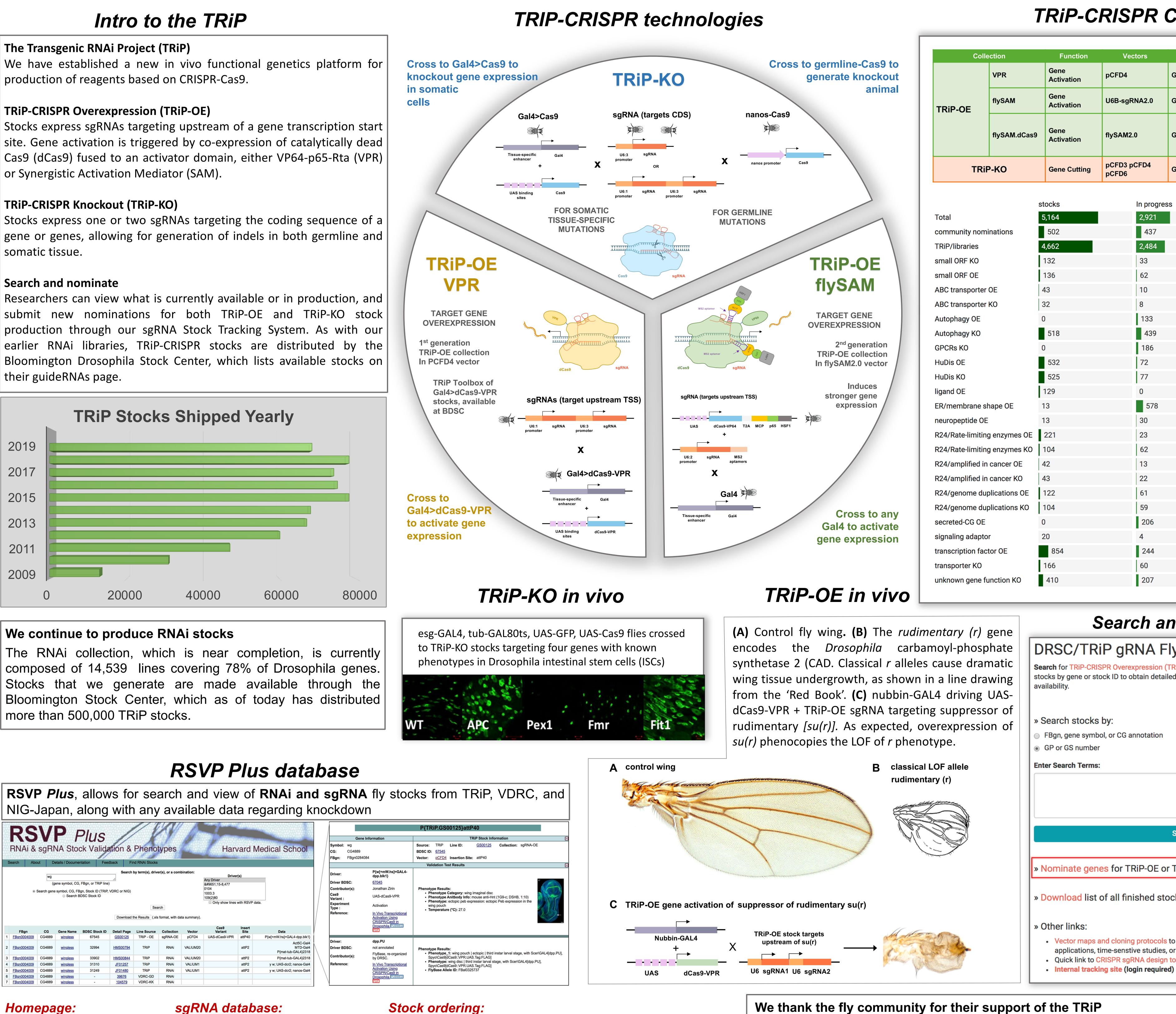# Large-scale transgenic Drosophila resource collections for loss- and gain-of-function studies

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Intro to the TRiP

The Transgenic RNAi Project (TRiP)
We have established a new in vivo functional genetics platform for production of reagents based on CRISPR-Cas9.

TRiP-CRISPR Overexpression (TRiP-OE)
Stocks express sgRNAs targeting upstream of a gene transcription start site. Gene activation is triggered by co-expression of catalytically dead Cas9 (dCas9) fused to an activator domain, either VP64-p65-Rta (VPR) or Synergistic Activation Mediator (SAM).

TRiP-CRISPR Knockout (TRiP-KO)
Stocks express one or two sgRNAs targeting the coding sequence of a gene or genes, allowing for generation of indels in both germline and somatic tissue.

Search and nominate
Researchers can view what is currently available or in production, and submit new nominations for both TRiP-OE and TRiP-KO stock production through our sgRNA Stock Tracking System. As with our earlier RNAi libraries, TRiP-CRISPR stocks are distributed by the Bloomington Drosophila Stock Center, which lists available stocks on their guideRNAs page.

TRiP Stocks Shipped Yearly

We continue to produce RNAi stocks
The RNAi collection, which is near completion, is currently composed of 14,539 lines covering 78% of Drosophila genes. Stocks that we generate are made available through the Bloomington Stock Center, which as of today has distributed more than 500,000 TRiP stocks.

TRIP-CRISPR technologies

Cross to Gal4>Cas9 to knockout gene expression in somatic cells

TRiP-KO

Cross to germline-Cas9 to generate knockout animal

FOR SOMATIC TISSUE-SPECIFIC MUTATIONS

FOR GERMLINE MUTATIONS

TRiP-OE VPR

TARGET GENE OVEREXPRESSION

1st generation TRiP-OE collection In PCFD4 vector

TRiP Toolbox of Gal4>dCas9-VPR stocks, available at BDSC

Cross to Gal4>dCas9-VPR to activate gene expression

TRiP-OE flySAM

TARGET GENE OVEREXPRESSION

2nd generation TRiP-OE collection In flySAM2.0 vector

Induces stronger gene expression

Cross to any Gal4 to activate gene expression

TRiP-CRISPR Collections

Collection		Function	Vectors	Cross to	Notes	Stocks
TRiP-OE	VPR	Gene Activation	pCFD4	GAL4+dCas9-VPR	Use with TRiP-CRISPR Toolbox	2128
	flySAM	Gene Activation	U6B-sgRNA2.0	GAL4+flySAM	Use with TRiP-CRISPR Toolbox	623
	flySAM.dCas9	Gene Activation	flySAM2.0	GAL4	flySAM2.0 lines contain both sgRNA and dCas9-activator	343
TRiP-KO		Gene Cutting	pCFD3 pCFD4 pCFD6	GAL4+Cas9	Use with TRiP-CRISPR Toolbox	2070

	stocks	In progress	total
Total	5,164	2,921	8,085
community nominations	502	437	939
TRiP/libraries	4,662	2,484	7,146
small ORF KO	132	33	165
small ORF OE	136	62	198
ABC transporter OE	43	10	53
ABC transporter KO	32	8	40
Autophagy OE	0	133	133
Autophagy KO	518	439	957
GPCRs KO	0	186	186
HuDis OE	532	72	604
HuDis KO	525	77	602
ligand OE	129	0	129
ER/membrane shape OE	13	578	591
neuropeptide OE	13	30	43
R24/Rate-limiting enzymes OE	221	23	244
R24/Rate-limiting enzymes KO	104	62	166
R24/amplified in cancer OE	42	13	55
R24/amplified in cancer KO	43	22	65
R24/genome duplications OE	122	61	183
R24/genome duplications KO	104	59	163
secreted-CG OE	0	206	206
signaling adaptor	20	4	24
transcription factor OE	854	244	1.1
transporter KO	166	60	226
unknown gene function KO	410	207	617

TRiP-KO in vivo

esg-GAL4, tub-GAL80ts, UAS-GFP, UAS-Cas9 flies crossed to TRiP-KO stocks targeting four genes with known phenotypes in Drosophila intestinal stem cells (ISCs)

WT   APC   Pex1   Fmr   Fit1

TRiP-OE in vivo

(A) Control fly wing. **(B)** The *rudimentary (r)* gene encodes the *Drosophila* carbamoyl-phosphate synthetase 2 (CAD. Classical *r* alleles cause dramatic wing tissue undergrowth, as shown in a line drawing from the 'Red Book'. **(C)** nubbin-GAL4 driving UAS-dCas9-VPR + TRiP-OE sgRNA targeting suppressor of rudimentary [su(r)]. As expected, overexpression of *su(r)* phenocopies the LOF of *r* phenotype.

A control wing

B classical LOF allele rudimentary (r)

C TRiP-OE gene activation of suppressor of rudimentary su(r)

Nubbin-GAL4 + UAS dCas9-VPR X TRiP-OE stock targets upstream of su(r) U6 sgRNA1 U6 sgRNA2

RSVP Plus database

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

Search and Nominate

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.

» Search stocks by:
○ FBgn, gene symbol, or CG annotation
● GP or GS number

Enter Search Terms:

[Search]

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

» Download list of all finished stocks (Last updated: 2019-03-17)

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