# The TRiP at HMS: tools for gene activation, repression and genome engineering

RNAi

CRISPR

DUAL SYSTEMS

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# *The Transgenic RNAi Project (TRiP)*

**Project began in 2008 to:**

1) **Develop** efficient vectors for RNAi

2) **Generate** a genome-scale collection of RNAi stocks with input from the community

3) **Distribut**e the lines as they are generated through existing stock centers

| TRiP RNAi Stocks | | | | | |
|---|---|---|---|---|---|
| Generation | Vector | Hairpin | # Stocks at BDSC | Target genes | Use in |
| 1st Generation | VALIUM1 | dsRNA | 678 | 534 | soma |
| | VALIUM10 | dsRNA | 1808 | 1753 | soma |
| 2nd Generation | VALIUM20 | shRNA | 9331 | 7418 | soma, germline |
| | VALIUM21 | shRNA | 96 | 96 | soma, germline |
| | VALIUM22 | shRNA | 1628 | 1455 | soma, germline |
| 3rd Generation | pNP | shRNA | 0 (Tsinghua, China) | 1093 | soma, germline |

Generated ~15,800 RNAi stocks

Currently, BDSC distributes 13,541 RNAi stocks, and to date have shipped over 770,000 TRiP stocks to 1,406 different user groups in 45 US states and 47 countries
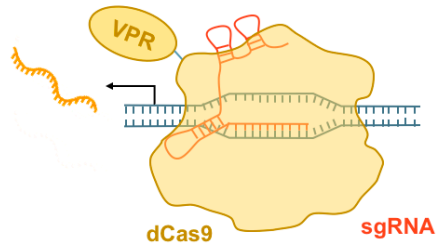
# TRiP-CRISPR knockout (TRiP-KO)
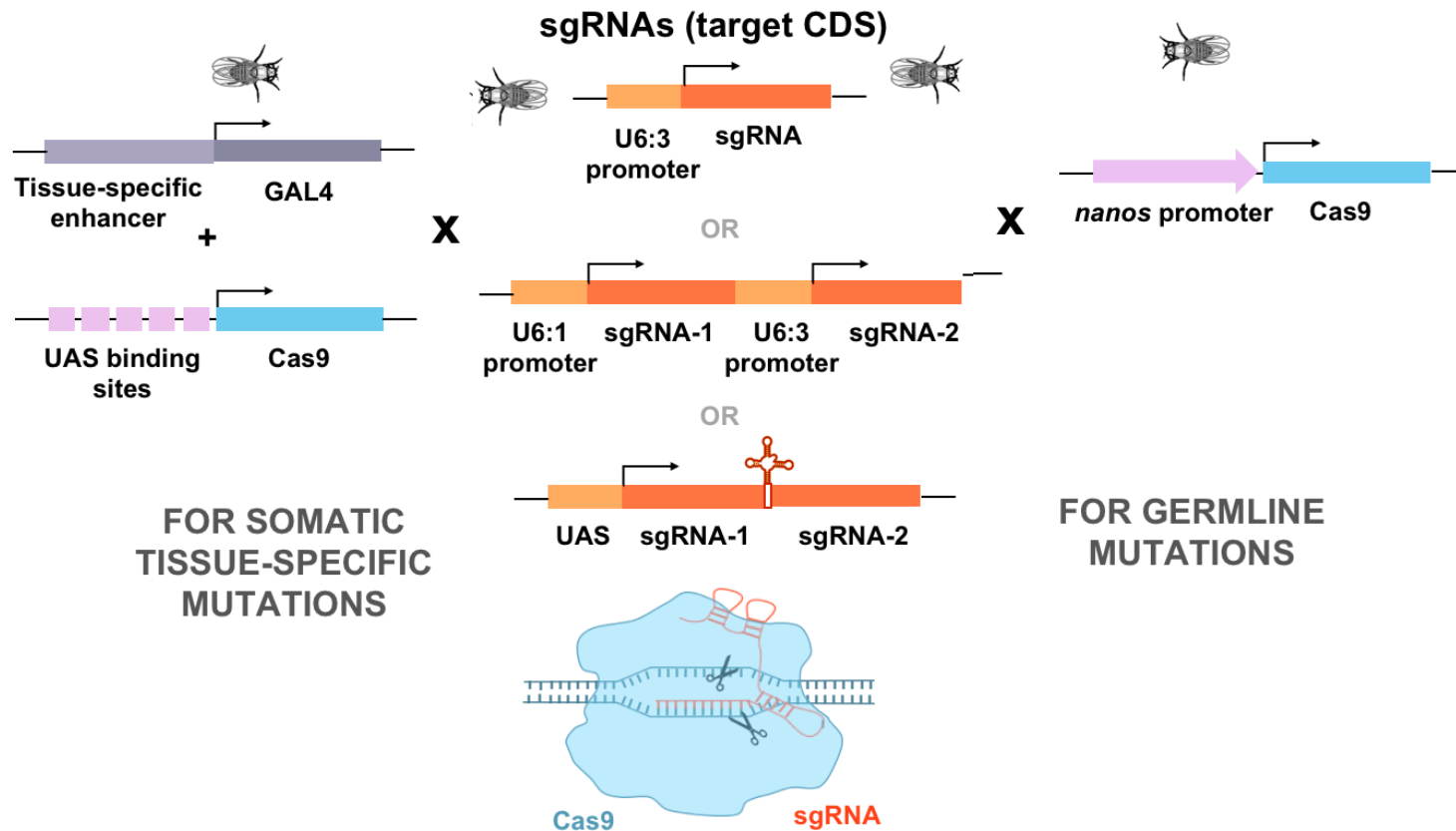


TRiP-KO stocks express one or two sgRNAs targeting the coding sequence of a gene or genes, allowing for generation of indels in both germline and somatic tissue
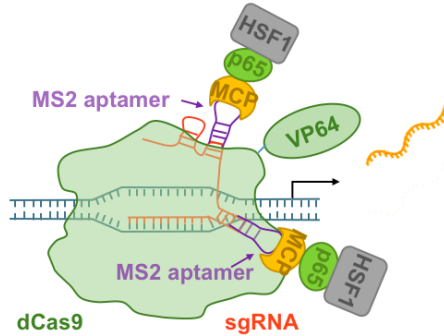
~2100 TRiP-KO lines targeting community nominations, orthologs of human diseases, and other focused gene sets

# TRiP-CRISPR overexpression (TRiP-OE)

## TRiP-OE VPR



dCas9    sgRNA

**sgRNAs (target upstream TSS)**



U6:1 promoter    sgRNA-1    U6:3 promoter    sgRNA-2

**X**

GAL4>dCas9-VPR



Tissue-specific enhancer    Gal4

**+**



UAS binding sites    dCas9-VPR

## TRiP-OE flySAM



HSF1    p65    MCP    VP64

MS2 aptamer

MS2 aptamer    MCP    p65    HSF1

dCas9    sgRNA

**sgRNA (targets upstream TSS)**



UAS    dCas9-VP64    T2A    MCP    p65    HSF1

**+**



U6:2 promoter    sgRNA    MS2 aptamers

**X**

GAL4



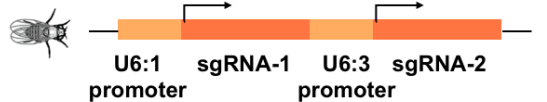Tissue-specific enhancer    Gal4

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TRiP-OE stocks express sgRNAs targeting genes upstream of the TSS. Gene activation is triggered by co-expression of catalytically dead dCas9 fused to an activator domai:
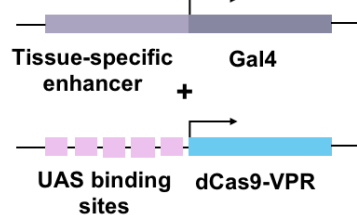
1st generation: VP64-p65-Rta (VPR)
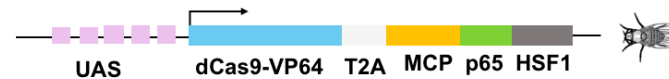
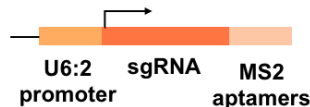2nd generation: Synergistic Activation Mediator (SAM)
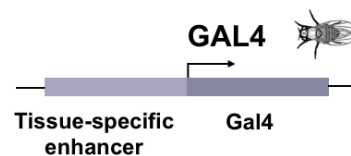
~3100 TRiP-OE lines targeting community nominations, orthologs of human diseases, and other focused gene sets

# sgRNA Fly Stock Database

Search TRiP-CRISPR stocks by gene identifier or by stock number

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

## fgr.hms.harvard.edu



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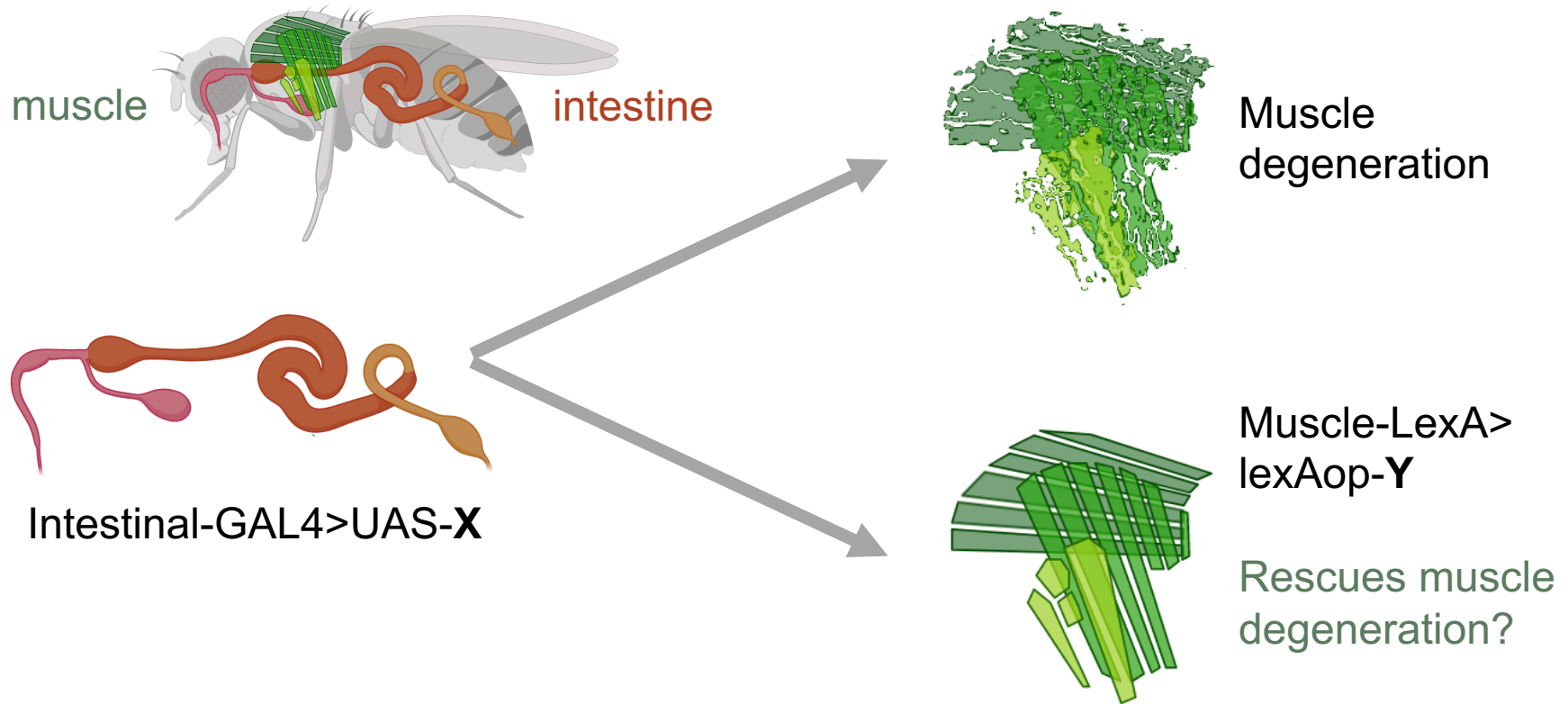
- Vector maps and cloning protocols to build your own constructs and flies for custom applications, time-senstive studies, or isoform-specific targets
- Quick link to CRISPR sgRNA design tool
- **Internal tracking site (login required)**

# What's in store from the TRiP?

Complete our coverage of TRiP RNAi and CRISPR fly stock resources for fly genes orthologous to genes known or suspected to be associated with human diseases

# What's in store from the TRiP?

**Dual binary systems**



muscle        intestine

Intestinal-GAL4>UAS-**X**

Muscle degeneration

Muscle-LexA>
lexAop-**Y**

Rescues muscle degeneration?

# What's in store from the TRiP?

Expand the Drosophila toolkit for dual binary systems

- Compare efficacy of the LexA/lexAop and QF/QUAS systems for gene knockdown

- Generate a compatible library of shRNA lines targeting conserved signaling pathways.

- Generate LexA or QF lines in genes with well-characterized tissue-specific GAL4 expression

- Generate a toolbox of combined LexA or QF + GAL4 driver fly stocks.

# *Acknowledgements*

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