DRSC & TRiP Functional Genomics Resources:

Drosophila Genomic Technologies, Bioinformatics Tools for Model Organism Data Mining & More

Leadership Team: Stephanie Mohr and Jonathan Zirin (Technologies), Claire Yanhui Hu (Bioinformatics), Norbert Perrimon (PI)
Staff: Roderick Brathwaite, Cooper Cavers, Ryan Colbeth, Colby Devereaux, Shannon Knight, Fengge Li, Lu-Ping Liu Jonathan Rodiger, Rong Tao, Sara VanNest, Eric Vogt, Donghui Yang-Zhou
Location: New Research Building Room 336, 77 Avenue Louis Pasteur, Boston, MA 02115
Affiliation: Department of Genetics | Blavatnik Institute, Harvard Medical School

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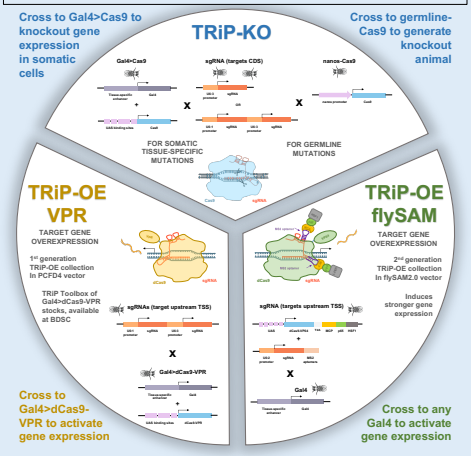
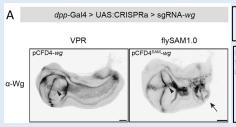
CRISPR Fly Stocks

- TRiP-CRISPR Overexpression collection (TRiP-OE) - for gene activation in somatic and germline cells
- TRiP-CRISPR Knockout collection (TRiP-KO) - for mutagenesis in somatic and germline cells
- TRiP-CRISPR Toolbox - for stage- and tissue-specific Cas9 expression





In vivo activation using CRISPRa

Ectopic activation of *wg* in the larval wing disc using VPR and flySAM. Arrow indicates duplicated wing pouch, arrowheads indicate ectopic Wg expression

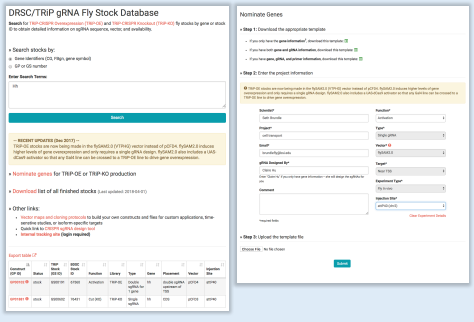
TRiP-CRISPR sgRNA Production

VPR flySAM single guide double guide

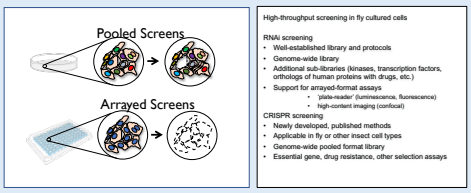
Overexpression Stocks	2,128	263
Overexpression In Progress	398	797
Knockout Stocks	1,857	296
Knockout In Progress	1,599	194

Community Access & Nominations

sgRNA Fly Stock Database (http://www.flyrnai.org/tools/grna_tracker/). Researchers can search TRiP-CRISPR stocks by gene identifier or by stock number and nominate genes for TRiP-OE or TRiP-KO production.



RNAi and CRISPR Screens in Fly Cells

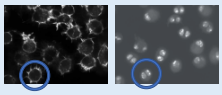


High-throughput screening in fly cultured cells

RNAi screening
- Well-established library and protocols
- Genome-wide library
- Additional sub-libraries (kinases, transcription factors, orthologs of human proteins with drugs, etc.)
- Support for arrayed-format assays
  - 'plate-reader' (luminescence, fluorescence)
  - high-content imaging (confocal)

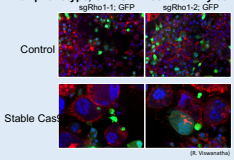
CRISPR screening
- Newly developed, published methods
- Applicable in fly or other insect cell types
- Genome-wide pooled format library
- Essential gene, drug resistance, other selection assays

Rho1 phenotype, RNAi knockdown in fly cells



RNAi screening workflow (>200 projects supported)

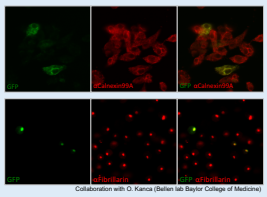
Rho1 phenotype, CRIPSR knockout in fly cells



CRISPR arrayed format screening in development
CRISPR pooled format screening available

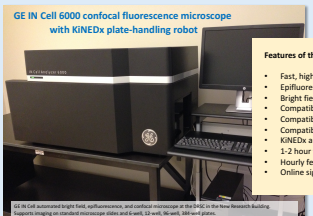
DRSC Provides
- CRISPR modified fly cell lines (knockouts, GFP tags) and protocols for cell line modification
- RNAi or CRISPR libraries
- Consultation at all stages
- Instrumentation for arrayed-format readouts
- Bioinformatics support

CRISPR Fly Cell Line Modification



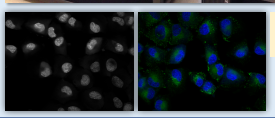
Expected localization	Target gene	Status
ER	Cnx99A	Visible, correct localization
Nucleolus	Fib	Visible, correct localization
Golgi (cis-Golgi)	Grasp	Visible, correct localization
Golgi (trans-Golgi)	Golgin245	Visible, correct localization
Centrosomes (mega foci)	Rab11	Visible, correct localization
Actin filaments/Actin cytoskeleton	Rab23	Visible, correct localization
Nuclear membrane	Lam	Visible, correct localization
ER (reticulum)	Sec61	Visible, correct localization
Kinetochore	polo	Visible, correct localization
Cytoskeleton	spn	Visible, correct localization
Mitochondria	Tom20	Visible, correct localization
Lysosomes	Arl8/Gie	Visible with anti-GFP_correct localization
Actin/Cytoskeleton	Zipper	Visible with anti-GFP_correct localization
Mitochondria	Tom17b	Visible, incorrect localization
Nuclear membranes, inner	LBR	Visible with anti-GFP_incorrect localization
G Bodies (cytoplasmic punctate)	Pfk	In progress
Endosomes, early	Rab5	In progress
Golgi (trans-Golgi)	Sec71	No GFP signal
Lipid droplets	Sepin	GFP signal?
Actin-cortex	dia	Failed at design stage
ER/cytosol, late	Rab7	Failed at design stage

GE IN Cell High-Content Imaging System



Features of the GE IN Cell 6000 at the DRSC

- Fast, high-quality imaging
- Epifluorescence or confocal imaging
- Bright field imaging with digital DIC option
- Compatible with microscope slides
- Compatible with 6-well, 12-well, etc. plates
- Compatible with 96-well & 384-well plates
- KiNEDx automated plate handling robot
- 1-2 hour training available
- Hourly fees for use after training
- Online sign-up for time

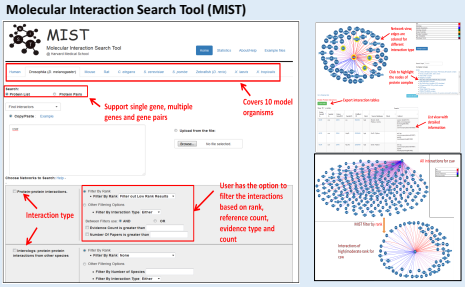


So many images! What to do? Image-based screens generate lots of data. We are working with the Image Data Management Core to use OMERO for image view, share, and figure-making, and with RITS to develop better image storage workflows.

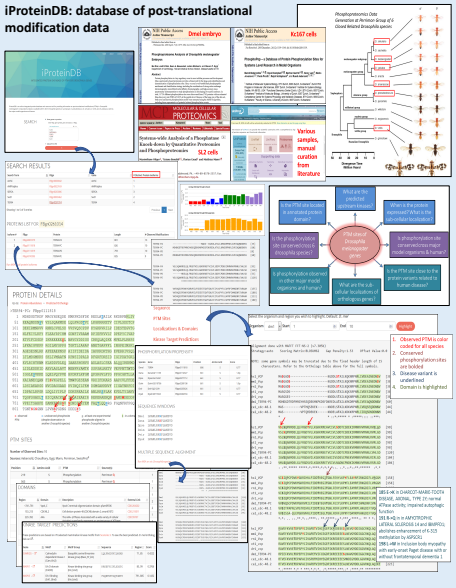
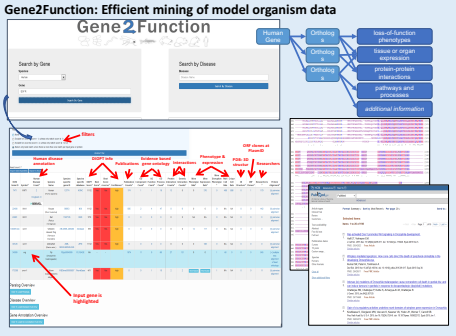
Bioinformatics Resources

We have developed a large suite of tools for bioinformatics applications relevant to research in flies, humans, and other species. For a full list, please visit the Online Tools section of our website, fgr.hms.harvard.edu. Below we highlight three new resources.

Molecular Interaction Search Tool (MIST)



iProteinDB: database of post-translational modification data



Gene2Function: Efficient mining of model organism data