

DRSC & TRiP Functional Genomics Resources:

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

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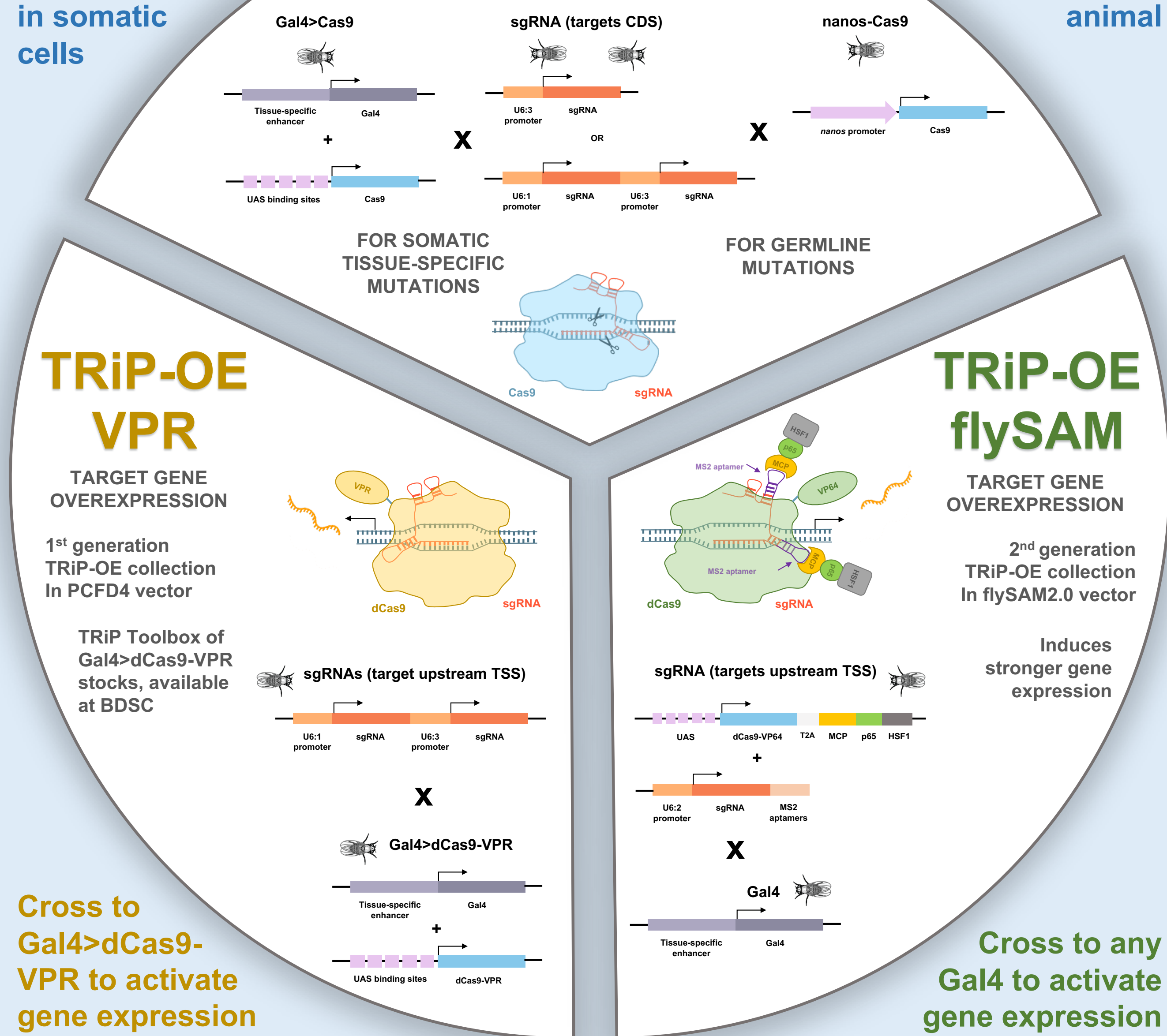
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CRISPR Fly Stocks

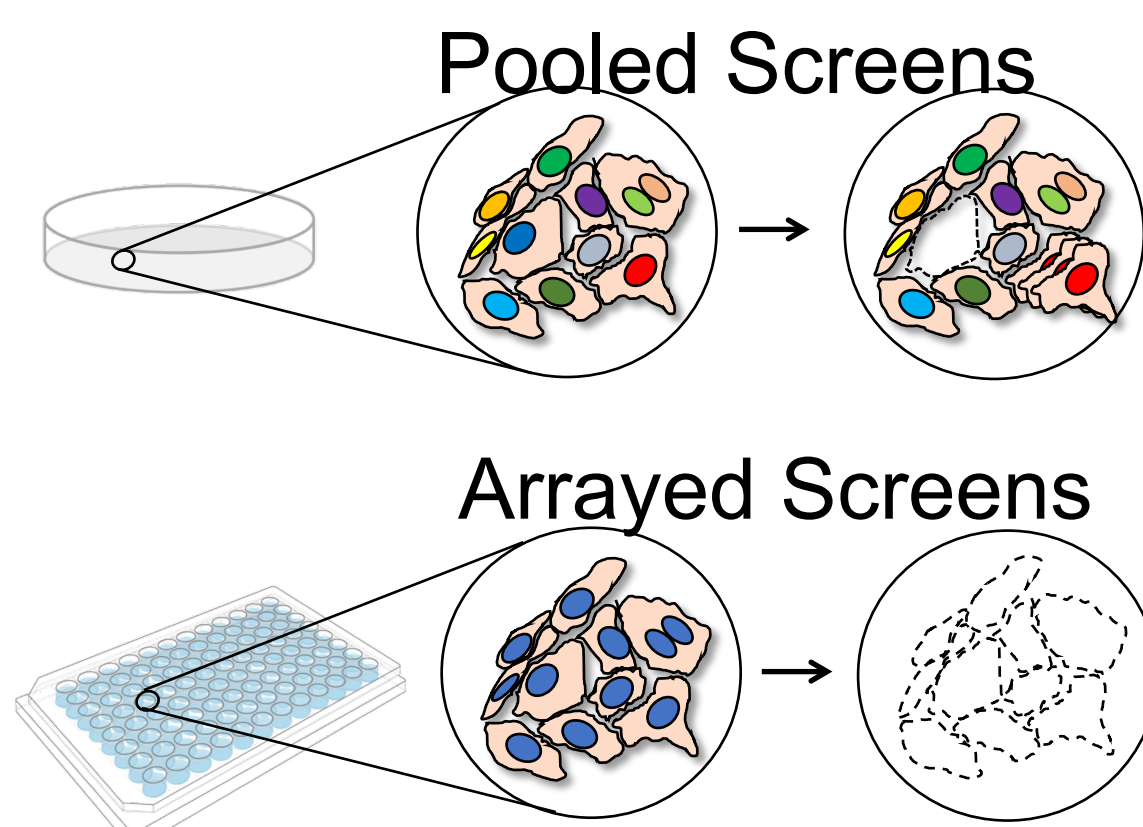
- TRIP-CRISPR Overexpression collection (TRIP-OE) - for gene activation in somatic and germline cells
- TRIP-CRISPR Knockout collection (TRIP-KO) - for mutagenesis in somatic and germline cells
- TRIP-CRISPR Toolbox - for stage- and tissue-specific Cas9 expression

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

Cross to germline-Cas9 to generate knockout animal



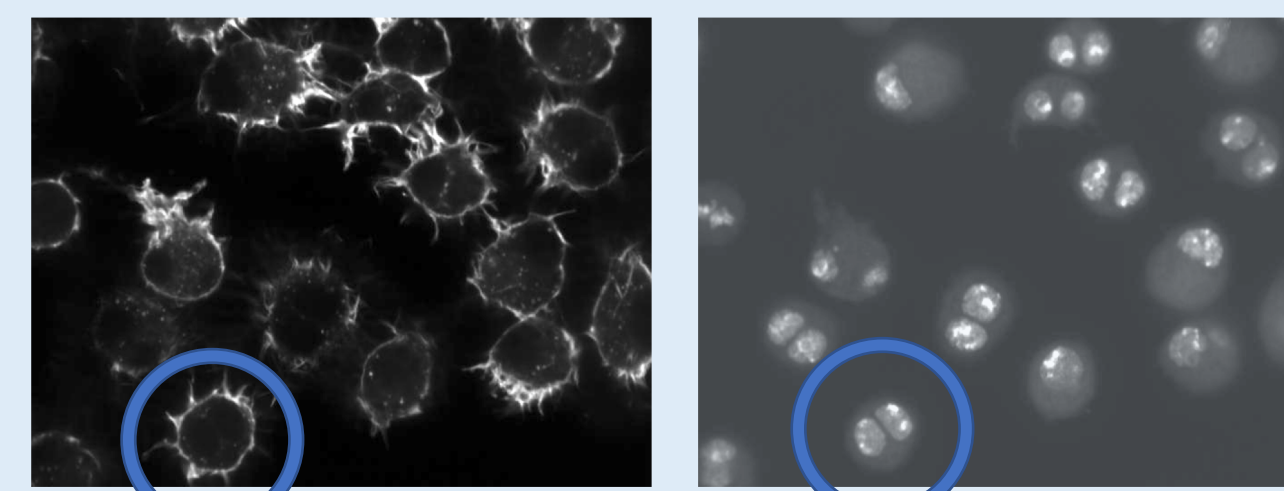
RNAi and CRISPR Screens in Fly Cells



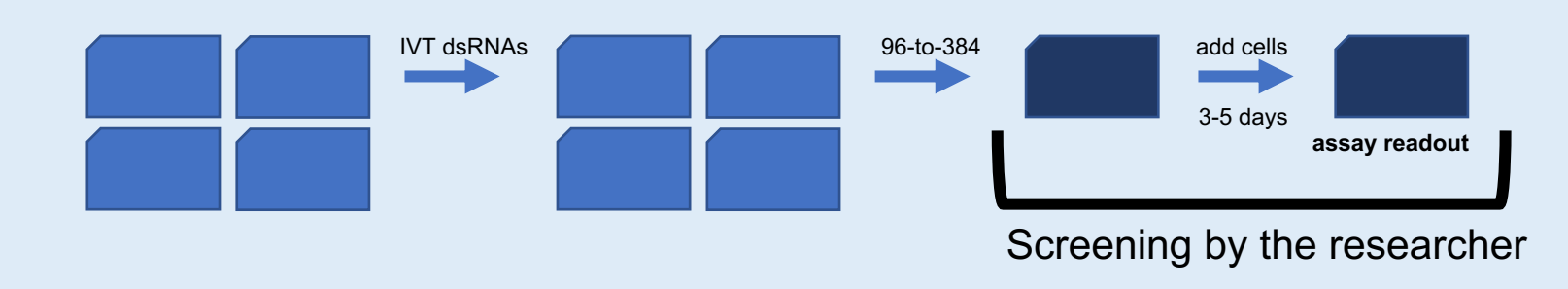
High-throughput screening in fly cultured cells

- 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

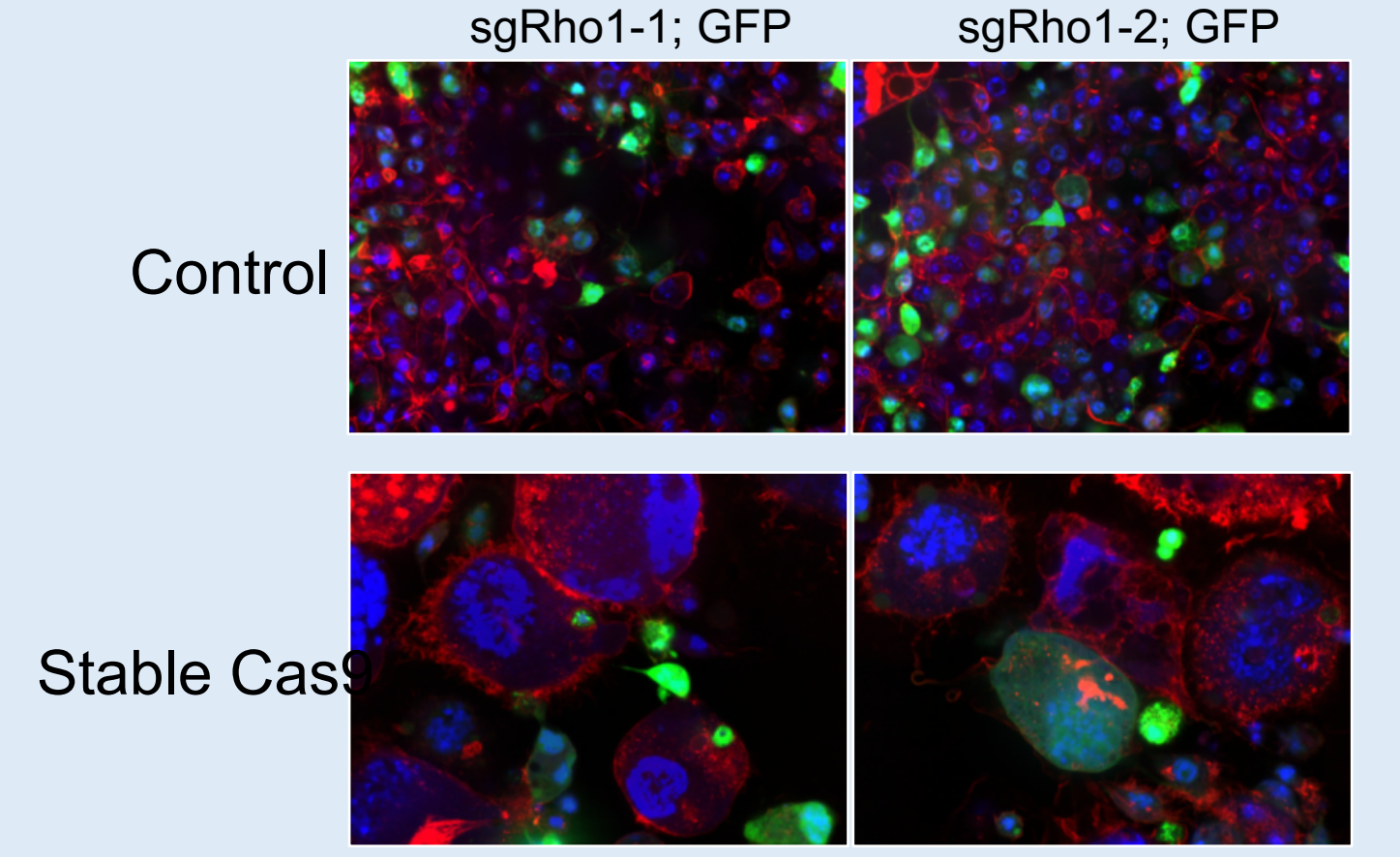
Rho1 phenotype, RNAi knockdown in fly cells



RNAi screening workflow (>200 projects supported)

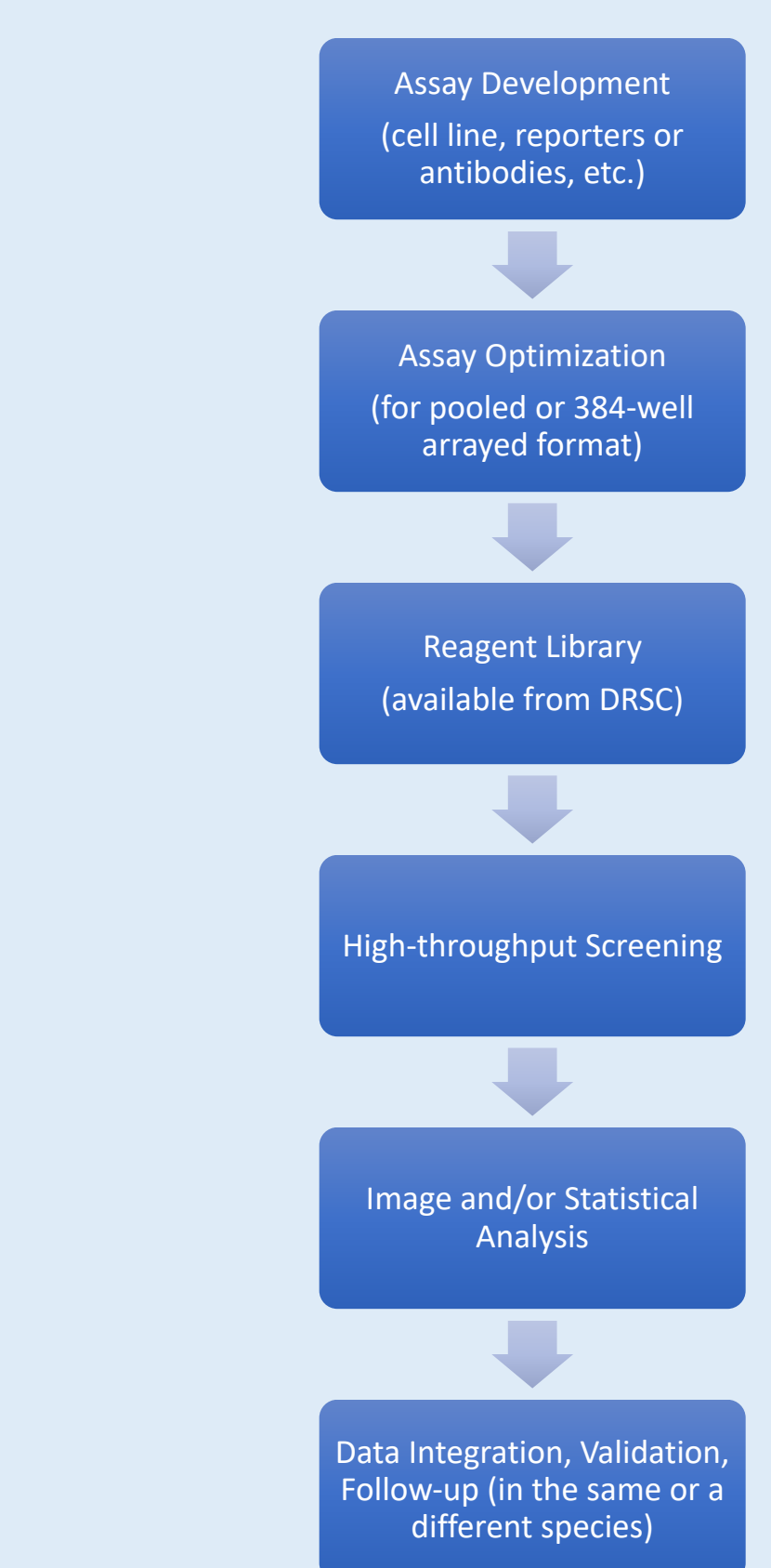


Rho1 phenotype, CRISPR knockout in fly cells



CRISPR arrayed format screening in development

CRISPR pooled format screening available



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

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)

MIST Molecular Interaction Search Tool

Support single gene, multiple genes and gene pairs. Covers 10 model organisms. User has the option to filter the interactions based on rank, reference count, evidence type and count.

iProteinDB: database of post-translational modification data

iProteinDB database of post-translational modification data. Includes search results, protein details, and various samples, manual curation from literature.

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/gma_tracker/). 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.

Search stocks by: Gene identifiers (CG, FBgn, gene symbol), GP or GS number.

Enter Search Terms: Fly ID.

Recent Updates (Dec 2017): TRIP-OE stocks are now being made in the flySAM2.0 (VTPMS) vector instead of pCFD4. flySAM2.0 induces higher levels of gene overexpression and only requires a single sgRNA 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: Select gene, Project, sgRNA, gRNA designed by, Experiment type, Hypothesis, Comment.

Download list of all finished stocks (last updated: 2018-04-01).

CRISPR Fly Cell Line Modification

Expected localization	Target gene	Status
ER	CoX5A	Visible, correct localization
Nucleolus	Fb	Visible, correct localization
Golgi (trans-Golgi)	Golp245	Visible, correct localization
Endosomes (recycling)	Rab11	Visible, correct localization
Autophagosomes/Aggregates	Rel2ZP	Visible, correct localization
Nuclear membrane	Lsm	Visible, correct localization
TR (trans-Golgi)	Sec23	Visible, correct localization
Kinetochore	plk0	Visible, correct localization
Lysosomes	sgln	Visible, correct localization
Mitochondria	Tim20D	Visible, correct localization
Autophagosomes	Atg18a	Visible with anti-GFP, correct localization
Mitochondria	Tim17b	Visible, incorrect localization
Nuclear membrane, inner	LBR	Visible with anti-GFP, incorrect localization
G-Bodies (cytoplasmic puncta)	Ypk	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
Endosomes, late	Rab7	Failed at design stage

GE IN Cell High-Content Imaging System

GE IN Cell 6000 confocal fluorescence microscope with KINEDx plate-handling robot

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

Example images: 60x objective 96-well plates. Left, DAPI. Right, DAPI (blue) and a marker of interest (green).

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.

Gene2Function: Efficient mining of model organism data

Gene2Function Efficient mining of model organism data. Search by Gene or Disease. Features include: Orthologs, loss-of-function phenotypes, tissue or organ expression, protein-protein interactions, pathways and processes, additional information.

Human Disease annotation, Evidence based Publications, Phenotype & expansion, ORF clones at PlasmiD, Research pgs, 3D structures, filter s.

Input gene is highlighted.