New technologies and resources from the Drosophila Research & Screening Center-Biomedical Technology Research Resource (DRSC-BTRR)

Stephanie Mohr, Muhammad Ahmad, Justin A. Bosch, Matt Butnaru, Adam Carte, Weihang Chen, Aram Comjean, Benjamin Ewen-Campen, Elizabeth Filine, Corey Foreman, Srishti Goswami, Myeonghoon Han, Yousuf Hashmi, Claire Yanhui Hu, Neha Joshi, Ah-Ram Kim, Lu-Ping Liu, Raphael Lopes, Enzo Mameli, William Mckenna, Karim Rahimi, Alexandria Risbeck, Emily Stoneburner, Raghuvir Viswanatha, Baolong Xia, Jonathan Zirin, Norbert Perrimon

Department of Genetics | Blavatnik Institute, Harvard Medical School Boston, MA, USA

View the poster online

Introduction

The DRSC-BTRR (NIH NIGMS GM132087), the current iteration of what was originally founded as the Drosophila RNAi Screening Center and Transgenic RNAi Project (DRSC/TRiP), focuses on the development, optimization, and dissemination of technologies for the benefit of the Drosophila and broader research communities.

Our areas of emphasis include:

(i) High-throughput cell-based screening in Drosophila and other insect cultured cells using CRISPR and other technologies

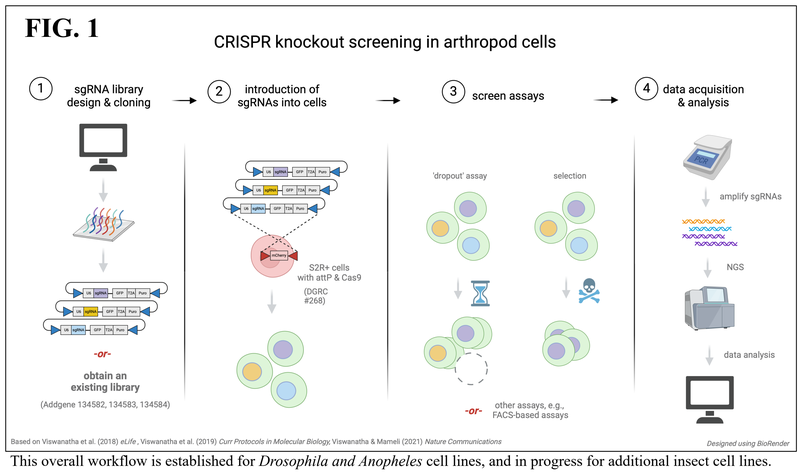
(ii) Molecular genetics and protein technologies for use in vivo in Drosophila or in other species (e.g., TRiP flies; see poster 604S)

(iii) Online resources for reagent design, ortholog identification, and other applications in common model species and a growing number of arthropod species (see below & talk 241)

With labs across the US & abroad, we engage in Driving Biomedical Projects (in-depth collaborations that assess and improve technologies for specific applications) and Collaboration & Service Projects (small projects) to ensure that our technologies meet real needs and are accessible. We also engage in outreach and training, including on-site visits, demo videos, and more.

Resource for discovery: CRISPR pooled cell screen technology

Context: Pooled-format CRISPR screens facilitate precision and large-scale screening without the need for automated equipment. We have robust methods for pooled CRISPR KO and CRISPRa screening in *Drosophila* cells, and for CRISPR KO in mosquito cells, e.g., *Anopheles* (Refs 1-4; **FIG 1**). **The overall approach is summarized in this graphic:**



This overall workflow is established for *Drosophila and Anopheles* cell lines, and in progress for additional insect cell lines.

Advantages of screening in pooled format in cultured cells:

- Relatively uniform cell type
- Easily scalable to comprehensive, genome-wide studies
- Identify high-confidence genes to test in *in vivo* assays

Limitations:

- Cell lines do not necessarily model cell type-specific behaviors
- Limited to query of cellular activities
- High-throughput studies associated with false discovery

"How to screen" info page

Addressing limitations:

- New cell lines from A. Simcox and colleagues provide better models of muscle, neuronal, blood, etc. cell types (Ref 5)
- We are working to expand applicable assay types, e.g., for study of pathways, host-microbe interactions, etc.
- We developed a 'version 2' of the *Drosophila* CRISPR KO sgRNA library and approach that has increased sensitivity
- CRISPR pooled screens are more specific, more sensitive, and lower cost than arrayed RNAi screens, and feasible in typical labs

Interested to screen? Contact stephanie_mohr@hms.harvard.edu for info

The diversity of topics addressed with screens so far includes:

Cell Signaling | ER stress | Bacterial Toxins | Hormone Transport

… and unpublished work on

- phagocytosis
- host-viral interactions
- cancer biology
- *and more*

New computational approach & online resource: FlyPredictome

Context:

- Identifying PPIs can associate an uncharacterized protein with a protein of known function ('guilt by association')
- Experimental methods for identifying PPIs are applicable at scale but associated with false discovery
- AlphaFold-Multimer (AFM) (Ref 6) can be used to predict PPIs
- Factors limiting broad use of AFM include missing real PPIs and high demand for computational time

What's new: We developed a new AFM-based score to improve computational prediction of PPIs, the "Local Interaction Score" or LIS (Ref 7), then used AFM-LIS to evaluate pairs from two large-scale experimental *Drosophila* PPI datasets: the FlyBi Y2H dataset (Ref 8) and the DPiM AP-MS dataset (Ref 9; **FIG 2**). These predictions and others, along with other available information (e.g., co-expression), are in our FlyPredictome online resource **(FIG 3).**

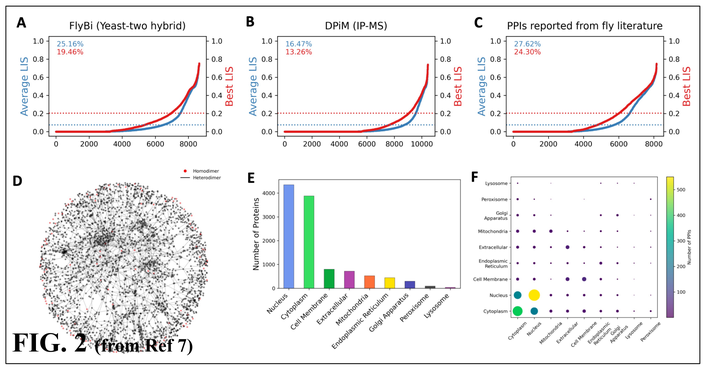


FIG. 2 (from Ref 7)

Fig. 2: Analysis of Drosophila PPI networks using large-scale proteomic data. From Ref 7. **A, B, C:** AFM-LIS scores for FlyBi Y2H, DPiM AP-MS, and literature-curated PPIs. **D**, Network visualization of predictions. **E**, Distribution of subcellular localization based on DeepLoc 2.0. **F**, Scatterplot of localization distribution.

https://www.flyrnai.org/tools/fly_predictome/

FlyPredictome Portal

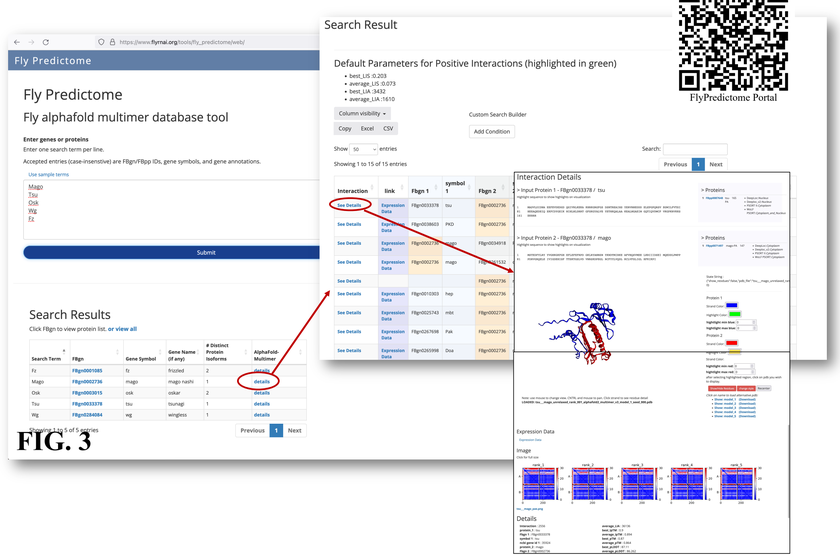


FIG. 3

Established technologies—*How will you use them?*

Technology or resource	How to access
Drosophila S2 cell 'GFP organelles' collection (ex. in **FIG. 4**)	DGRC in Bloomington, IN
Drosophila S2 cell 'tumor suppressor KO' collection	DGRC in Bloomington, IN
Drosophila CRISPR KO screen materials (Ref 2)	DGRC (cells), Addgene (libraries)
Mosquito CRISPR KO screen materials (Ref 4)	DGRC (cells), DRSC-BTRR (library)
Drosophila CRISPR activation screen materials (Ref 3)	DRSC-BTRR at HMS (us!)
DRSC Online Tools (Ref 11 and **FIG. 5**)	fgr.hms.harvard.edu/tools
CRISPR sgRNA designs for Drosophila	www.flyrnai.org/crispr3
PANGEA gene enrichment resource (multiple species supported)	www.flyrnai.org/tools/pangea
DIOPT (ortholog search tool—our most popular online resource)	www.flyrnai.org/diopt
FlyPrimerBank (qPCR primer resource)	www.flyrnai.org/flyprimerbank
Review – How to find info about 'unknown' genes	Ref 12

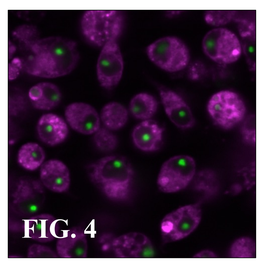


FIG. 4

Online Tools summary page

FIG. 5

More from our group: Posters 604S, 608S, 619S & Talk 241

References

1) Viswanatha et al. (2018) **Pooled genome-wide CRISPR screening for basal and context-specific fitness gene essentiality in Drosophila cells**. *eLife*.

2) Viswanatha et al. (2019) **Pooled CRISPR Screens in Drosophila Cells**. *Curr. Protoc. Mol. Bio*.

3) Xia et al. (2023) **Pooled genome-wide CRISPR activation screening for rapamycin resistance genes in Drosophila cells.** *eLife*.

4) Viswanatha, Mameli et al. (2021) **Pooled genome-wide CRISPR screening for basal and context-specific fitness gene essentiality in Drosophila cells.** *Nat. Comm*.

5) Coleman-Gosser, et al. (2023) **Continuous muscle, glial, epithelial, neuronal, and hemocyte cell lines for Drosophila research.** *eLife*.

6) Evans et al. (2021) **Protein complex prediction with AlphaFold-Multimer.** *BioRxiv*.

7) Kim et al. (2024) **Enhanced Protein-Protein Interaction Discovery via AlphaFold-Multimer**. *BioRxiv*.

8) Tang et al. (2023) **Next-generation large-scale binary protein interaction network for *Drosophila melanogaster*.** *Nat. Comm*.

9) Guruharsha et al. (2011) **A Protein Complex Network of *Drosophila melanogaster***. *Cell*.

10) Kanca et al. (2019) **An efficient CRISPR-based strategy to insert small and large fragments of DNA using short homology arms.** *eLife*.

11) Hu et al. (2021) **FlyRNAi.org-the database of the Drosophila RNAi screening center and transgenic RNAi project: 2021 update.** *Nucleic Acids Research*.

12) Mohr et al. (2023) **Finding information about uncharacterized Drosophila genes.** *Genetics*.

Funding

Major funding includes NIH NIGMS P41 GM132087 to NP and SM. Past funding includes R24 OD019847 to NP, SM & A. Simcox. NP is an investigator of Howard Hughes Medical Institute.