Development of CRISPR knockout screening in insect cell-lines

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1 Pooled format screening had been unavailable in Drosophila and invertebrates

	Mammalian cell-lines	Fly cell-lines	Other invertebrate cell-lines
Arrayed format	siRNA	dsRNA	
Pooled format	shRNA CRISPR loss-of-function CRISPR gain-of-function ORF overexpression	This study	

Figure 1. Despite the utility of CRISPR screening to basic cell biology in mammalian cell-lines, no protocol had been available for pooled screening in insect cell-lines.

2 A protocol for pooled CRISPR guide delivery in insect cell-lines

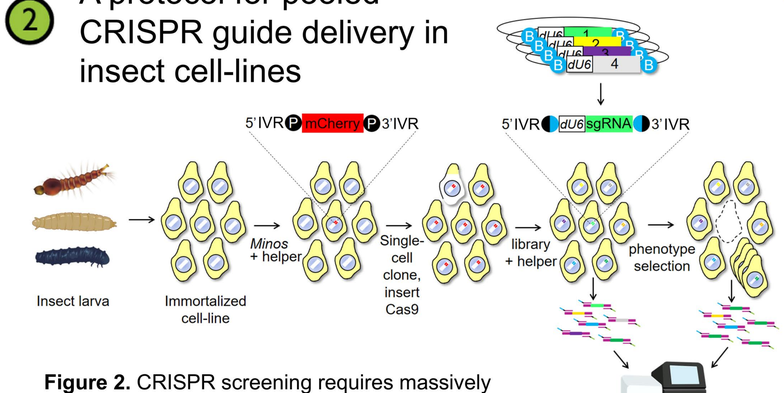


Figure 2. CRISPR screening requires massively parallel delivery of a library of sgRNA expression vectors into a large number of cells such that ~1000 cells receive each sgRNA. To do this, we used cells with an attP-mCherry-attP Minos cassette. Recombination-mediated cassette exchange (RMCE) is used to deliver the library. Cells are then subjected to phenotypic selection. CRISPR guide sequencing and analysis are used to determine genotype-phenotype relationships

3 Genome-wide CRISPR screen identifies ~1200 essential genes in Drosophila S2R+ cells

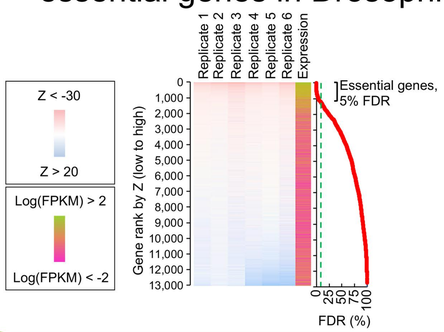


Figure 3. Genes that are essential in CRISPR screens yield more negative Z scores. Strikingly, genes with low Z scores are also identified by RNAseq as expressed genes. This relationship can be used to determine the false-discovery rate (FDR).

4 New insights into insect cell biology from context-dependent pooled CRISRP screens

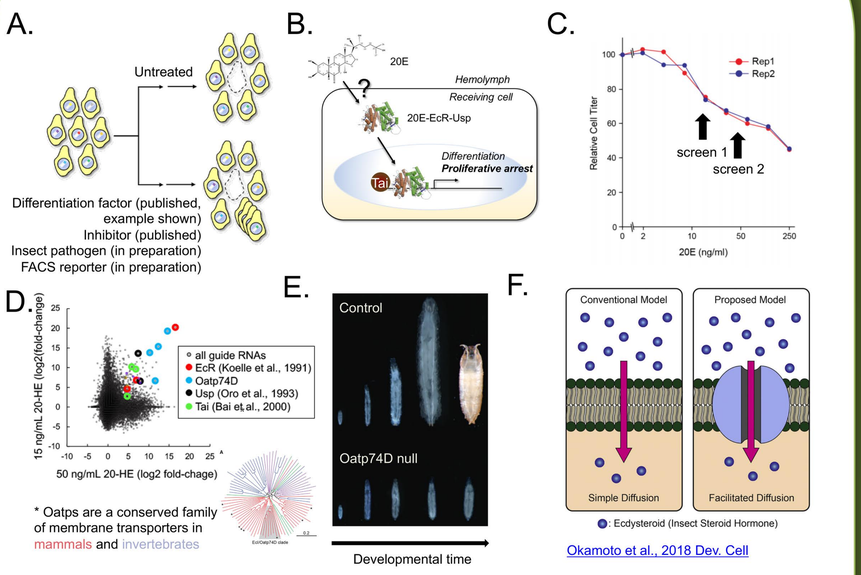


Figure 4. (A) Growth perturbing reagents or fluorescent readouts can be used in genome-wide screens. (B) Insect molting hormone, 20-Hydroxyecdysone (20E), is known to activate nuclear hormone receptor signaling which results in proliferative arrest, but how it enters cells is unknown. (C,D) A CRISPR screen for continued proliferation after 20E-induced arrest identified a novel component, *Oatp74D*, which is a transporter (E,F) *Oatp74D* is necessary for cellular uptake of 20E and normal development in *Drosophila*.

5 Pooled CRISPR screening pilot in mosquito cells (*Anopheles* Sua-5B)

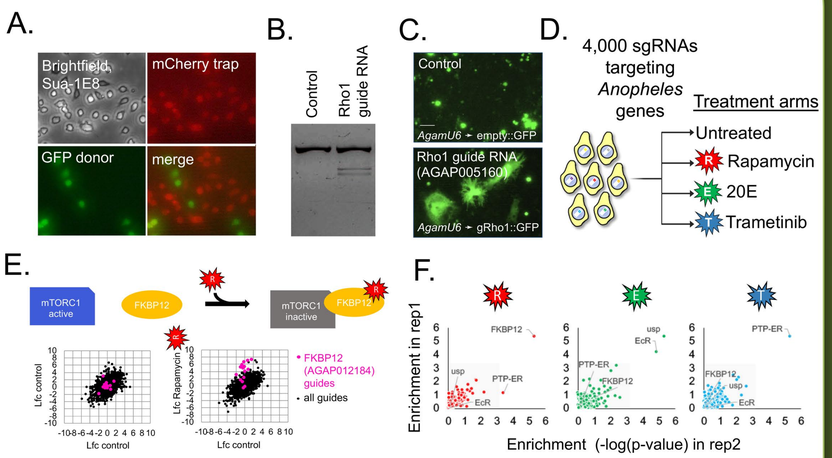


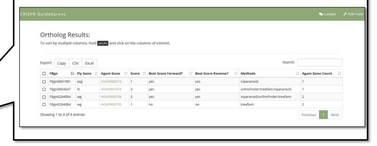
Figure 5. (A) Creation of RMCE-capable line, Sua-1E8. (B,C) *Anopheles* U6 promoter-driven sgRNAs allow robust KO in Sua cells. (D-F) A 4,000-sgRNA library was subjected in parallel to four treatment conditions. Pilot experiments positively selected the mosquito orthologs of known resistance factors for rapamycin (mTor inhibitor), 20E, or trametinib (MAPKK inhibitor).

6 Online resource for mosquito orthology prediction and CRISPR library design

CRISPR GuideXpress

https://www.flyrnai.org/tools/fly2mosquito/web/

Fly-to-mosquito orthology prediction using the DIOPT (multiple algorithm comparison) approach



FindCRISPRs tool for *A. gambiae* with batch search for designing CRISPR screening libraries

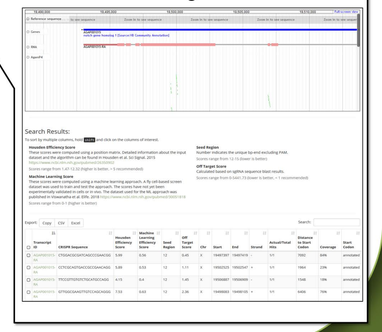


Figure 6. Introducing CRISPR GuideXpress for mosquitos. This tool allows orthology prediction from Drosophila to Anopheles as well as prediction of CRISPR guides for Anopheles singly or in batch. Other mosquito species will be added soon.

7 Conclusions and future directions

- Using a modified delivery protocol, CRISPR screens can be conducted in Drosophila cell-lines. These have the advantage of lower cost and lower false-discovery compared with existing approaches. This system can provide fresh insights into old problems, such as revealing a new essential component of insect molting hormone signaling.
- Pooled CRISPR screening offers the possibility of bringing high quality functional genomics to many additional insect species. We show that it is now possible to conduct similar screens in *Anopheles* mosquito cell-lines.
- In the future, we will continue to devise new screening strategies using the existing CRISPR screening reagents in Drosophila and *Anopheles*. We will also expand *Anopheles* screens genome-wide and attempt the method with other mosquito species.

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