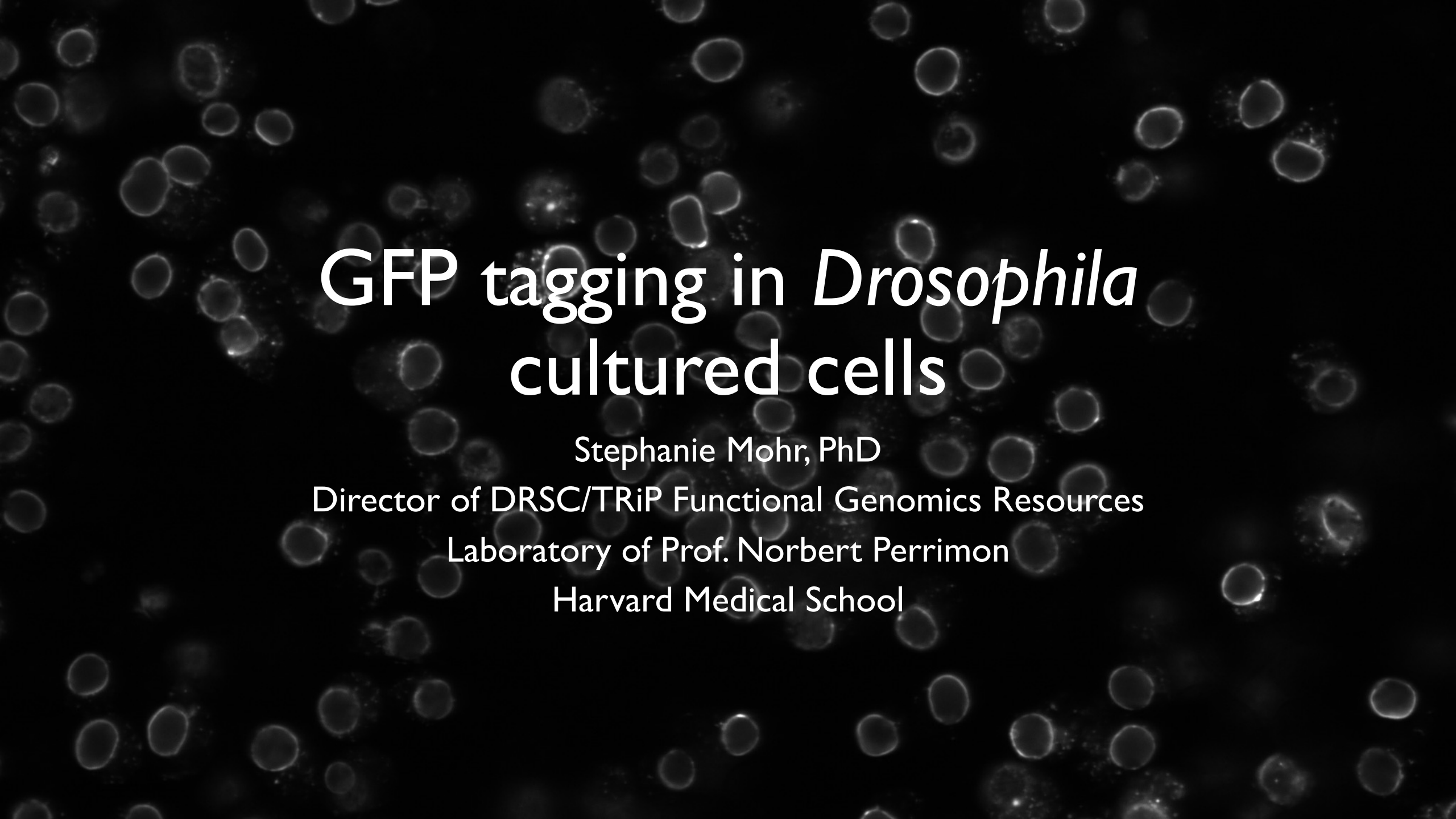


A black and white fluorescence microscopy image showing numerous Drosophila cultured cells. The cells are mostly round and appear to have a bright, glowing outline, likely due to GFP expression. They are distributed across the entire frame against a dark background.

GFP tagging in *Drosophila* cultured cells

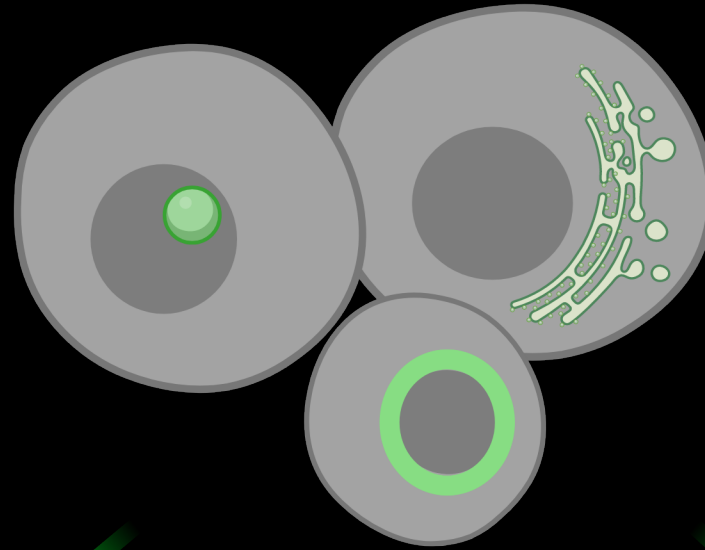
Stephanie Mohr, PhD

Director of DRSC/TRiP Functional Genomics Resources

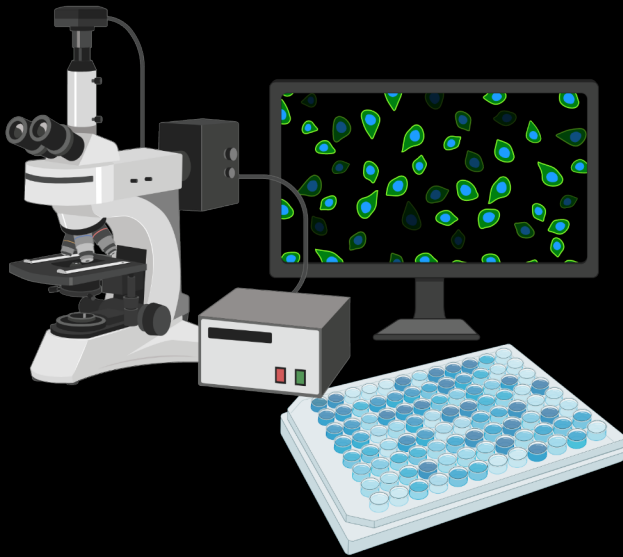
Laboratory of Prof. Norbert Perrimon

Harvard Medical School

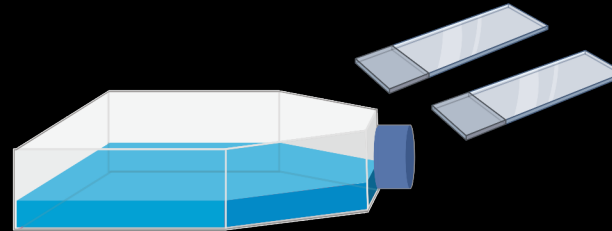
Why fluorescent protein tags?



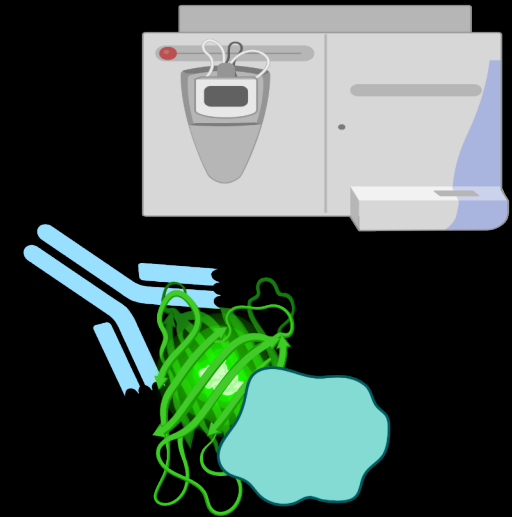
- Sub-cellular distribution
- Endogenous levels



High-Content Image-based Screens
(no antibodies, live cell)



Detailed Cell Biological Studies
(live cell, time lapse)

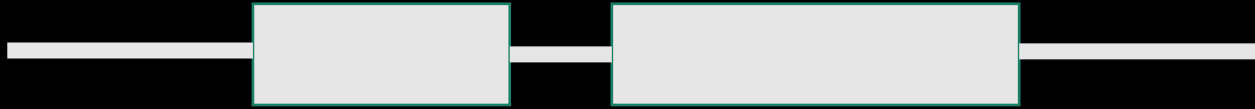


Proteomics Assays
(anti-GFP reagents)

CRISPR knock-in approaches

- plasmid-based donors, long homology arms
- single-stranded DNA donors, short homology arms
- 'armless' donors (CRISPaint method)

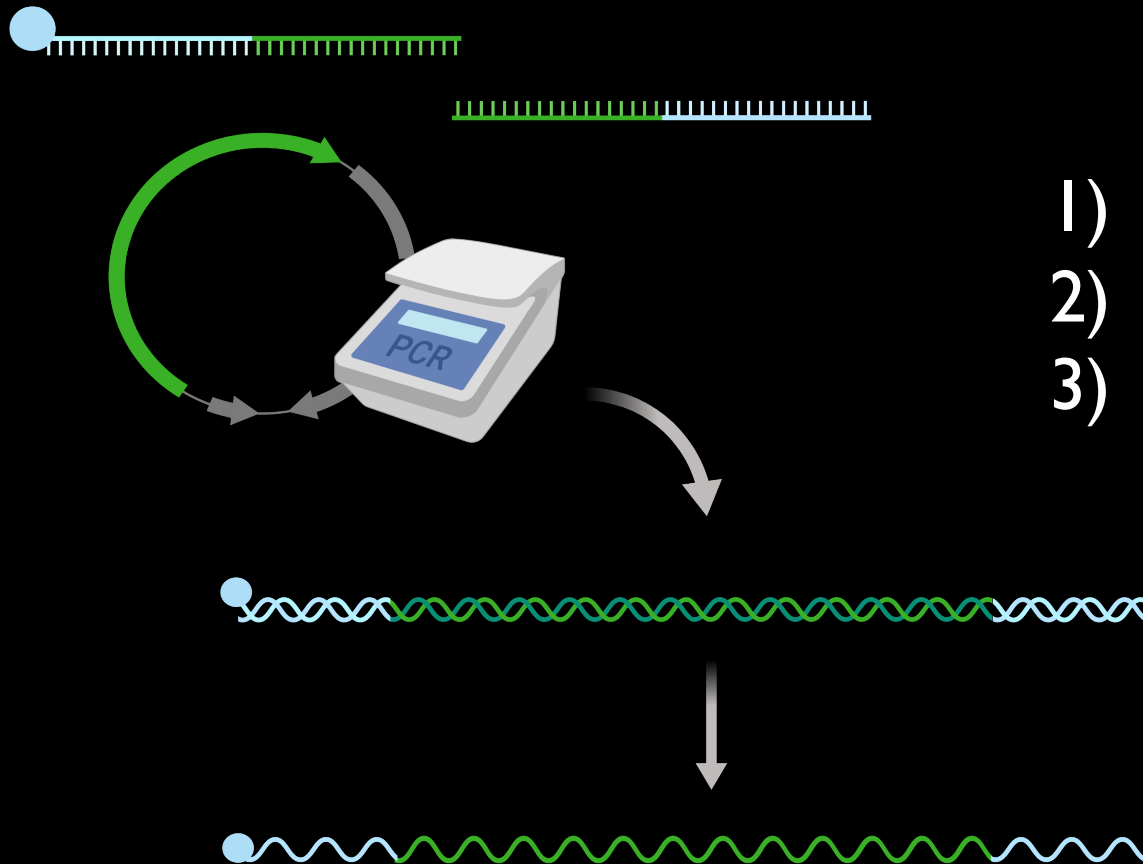
- *Efficient*
- *Easy to build the donor*
- *Artificial exon-based approach*



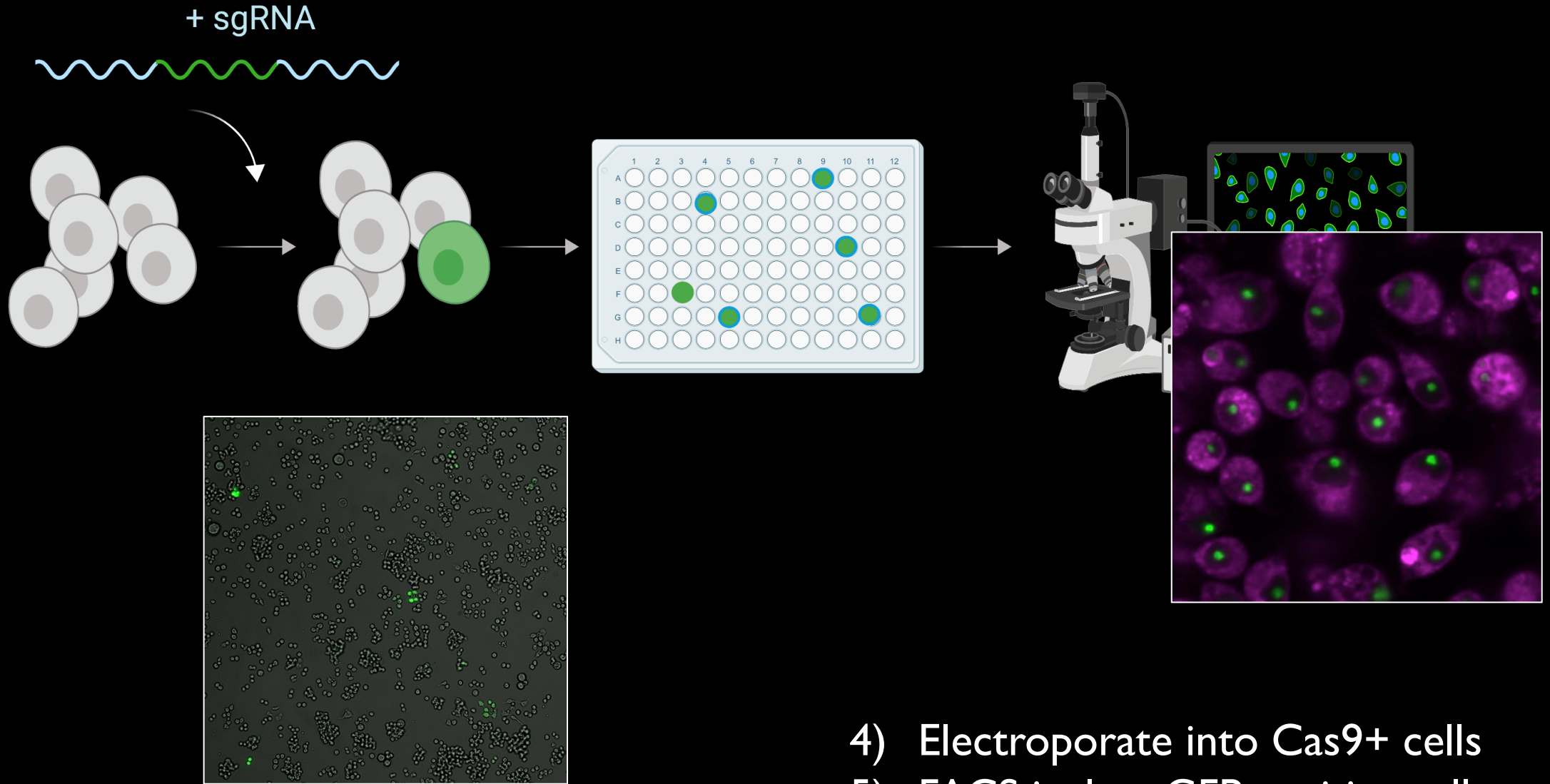
ssDNA artificial exon



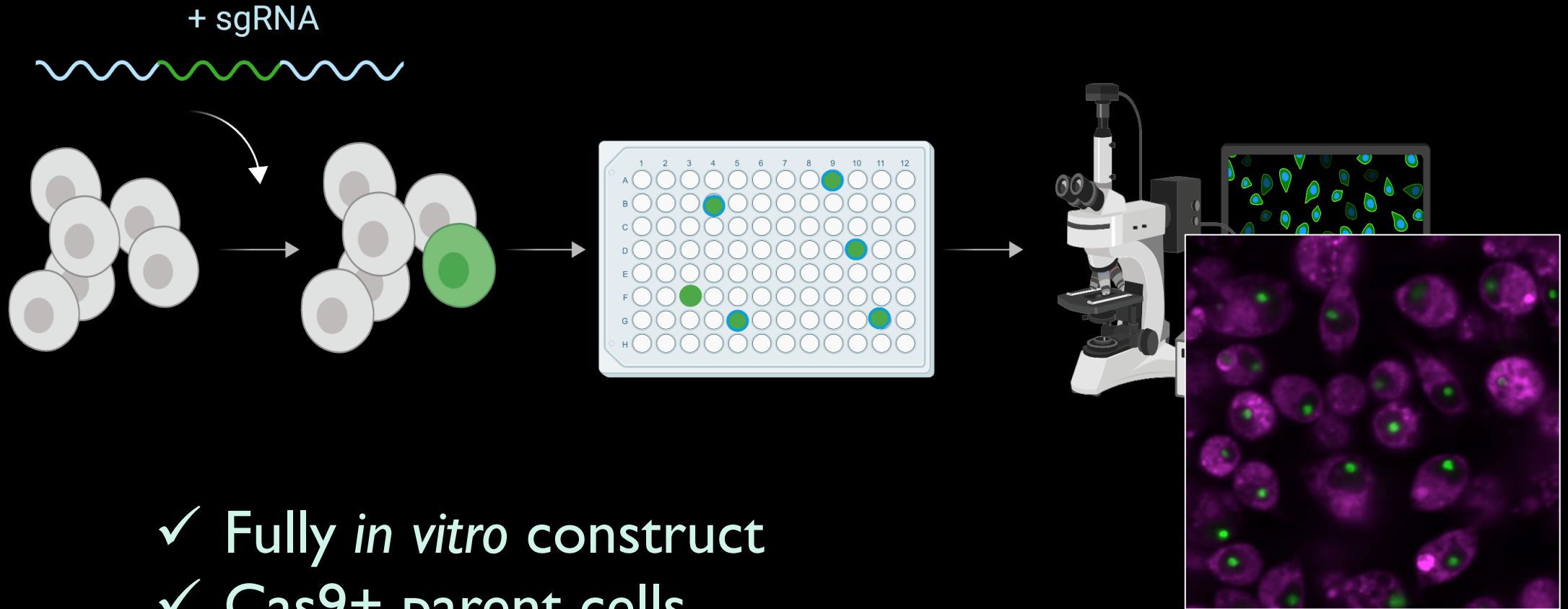
ssDNA synthesis



- 1) Oligos with short homology arms
- 2) PCR amplify GFP template
- 3) Digest to make ssDNA



- 4) Electroporate into Cas9+ cells
- 5) FACS isolate GFP positive cells
- 6) Imaging and molecular analysis



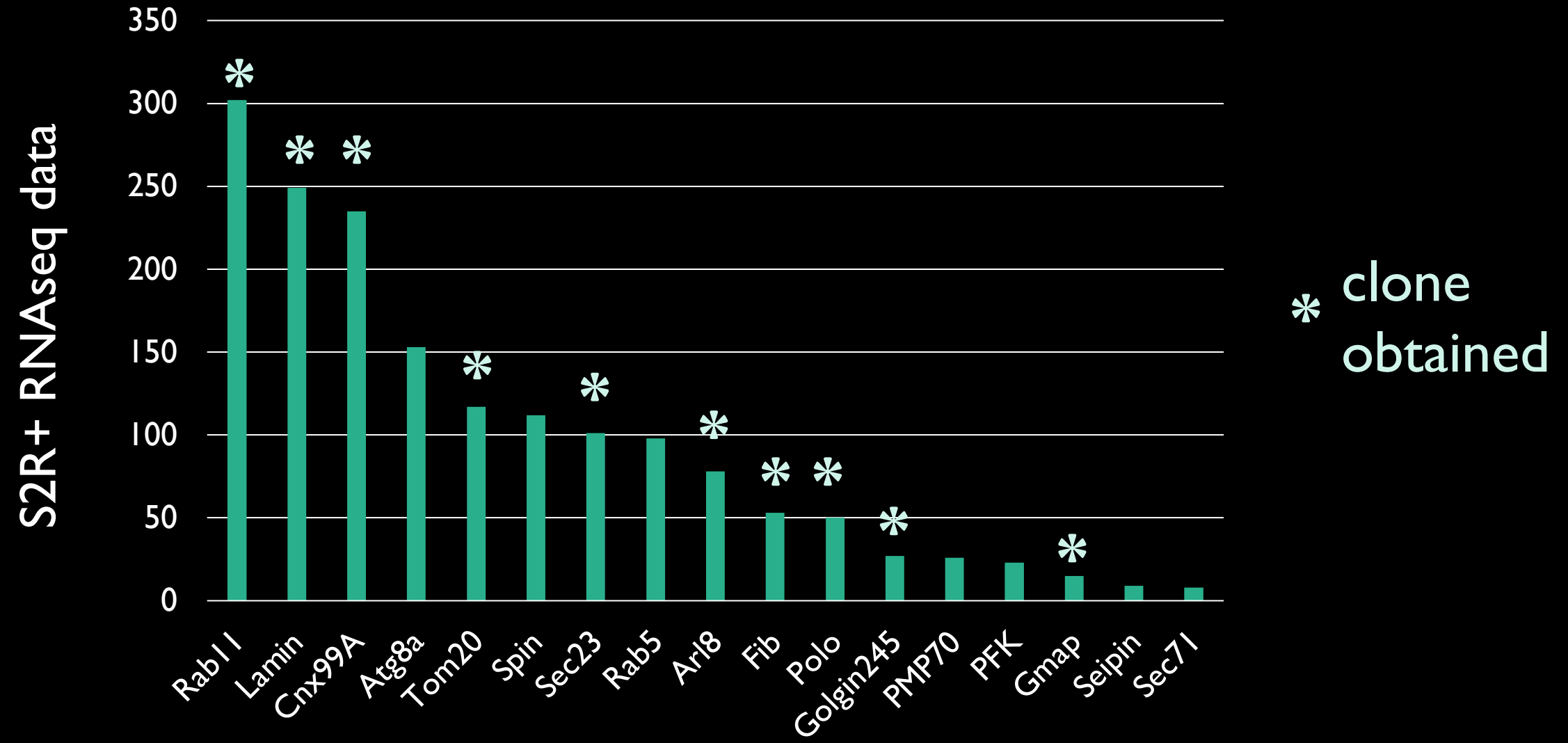
- ✓ Fully *in vitro* construct
- ✓ Cas9+ parent cells
- ✓ Electroporation
- ✓ Conditioned Media

Successfully tagged organelles

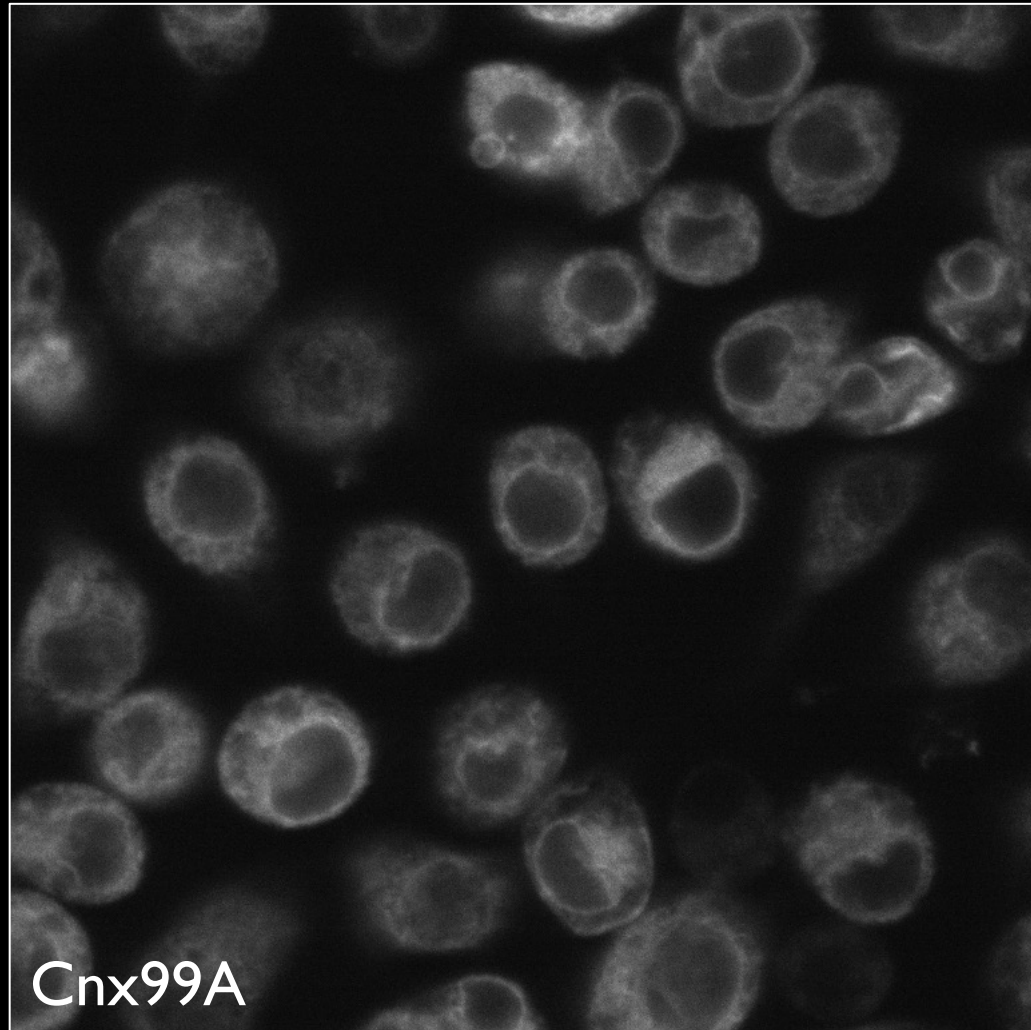
Organelle/Compartment	Protein targeted	Confirmed by Ab co-stain
Endoplasmic reticulum	Cnx99A	Yes
Endoplasmic reticulum (transitional)	Sec23	
Endosomes, recycling	Rab11	
Golgi (cis-Golgi)	Gmap	Yes
Golgi (trans-Golgi)	Golgin245	Yes
Kinetochore	Polo	
Lysosomes	Arl8	Yes
Mitochondria	Tom20	Yes
Nuclear membrane (inner)	Lamin	Yes
Nucleolus	Fibrillarin	Yes

Community Access:
Drosophila Genome
Resource Center (DGRC)
cell line repository in
Bloomington, IN

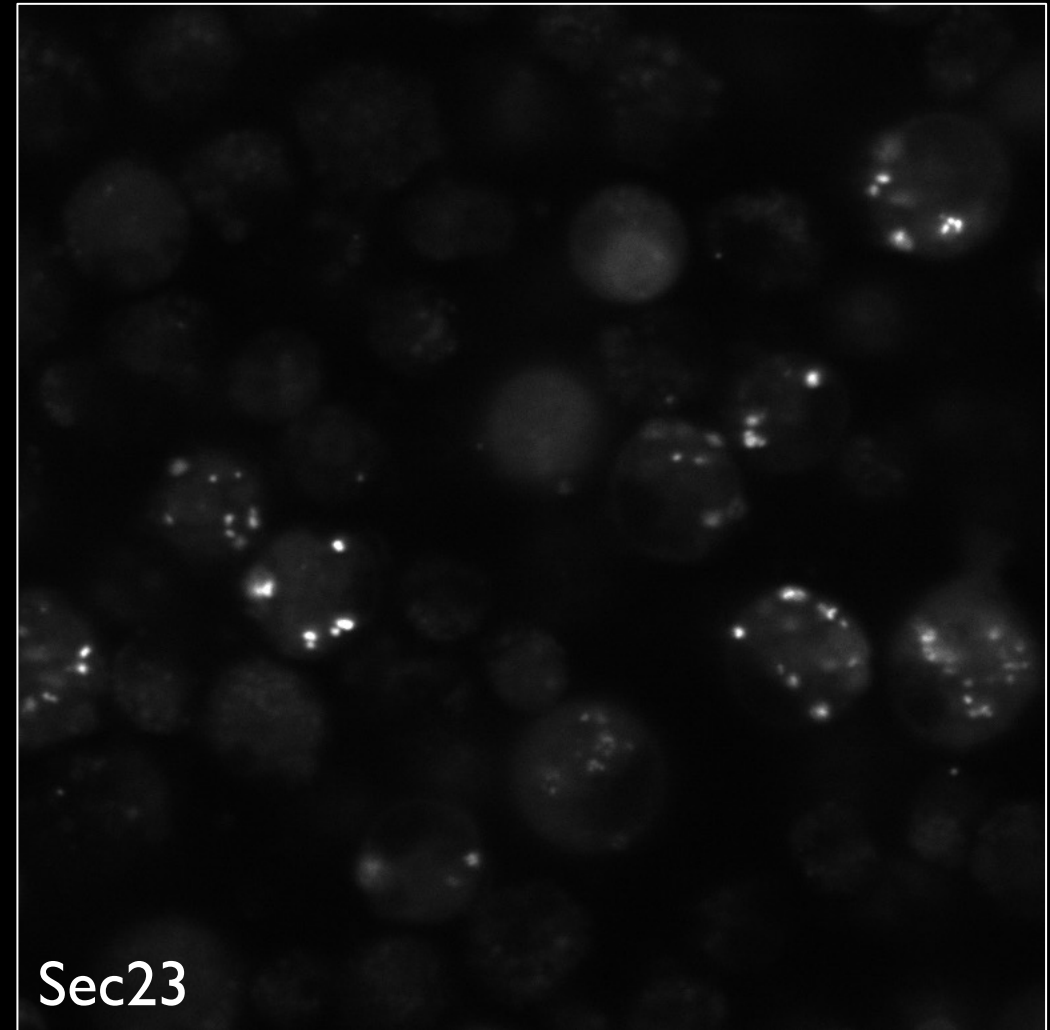
Expression vs. clone obtained



GFP live images

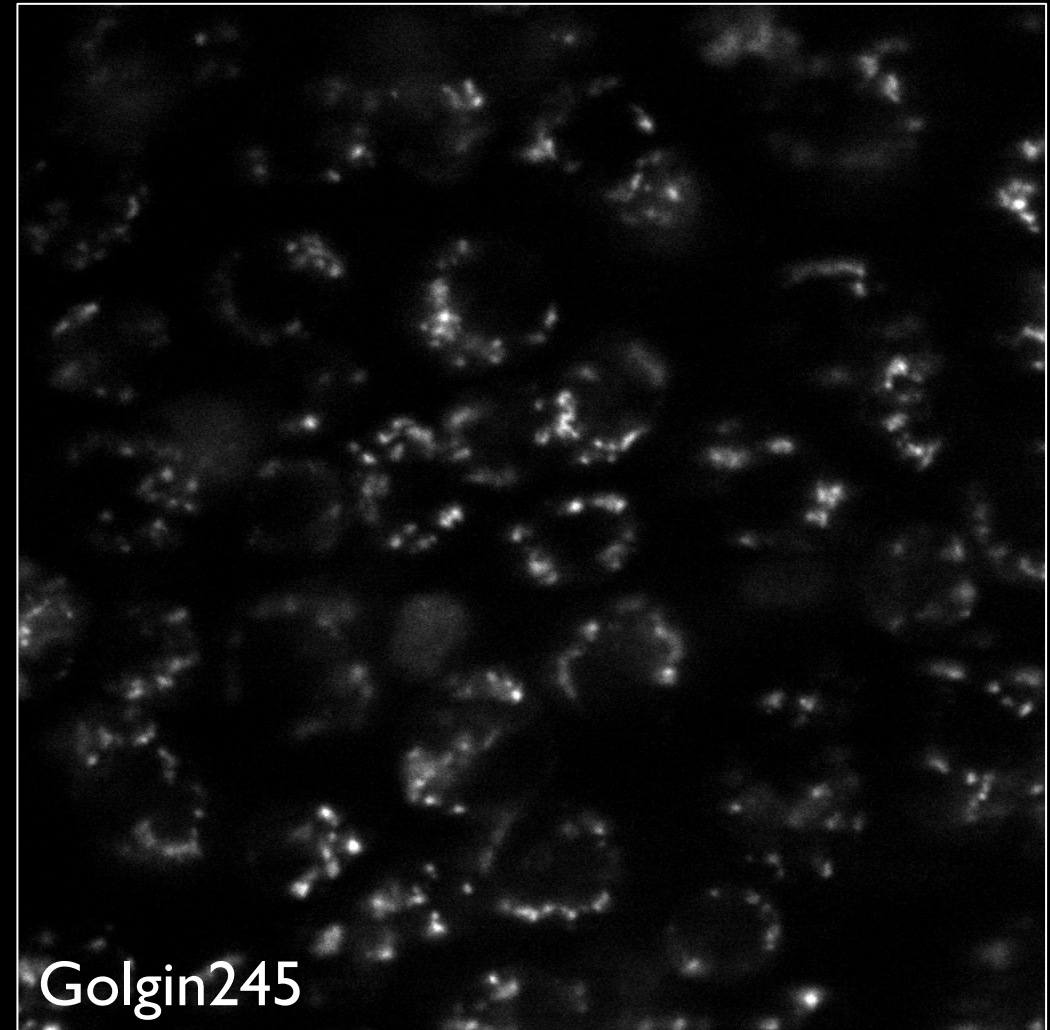
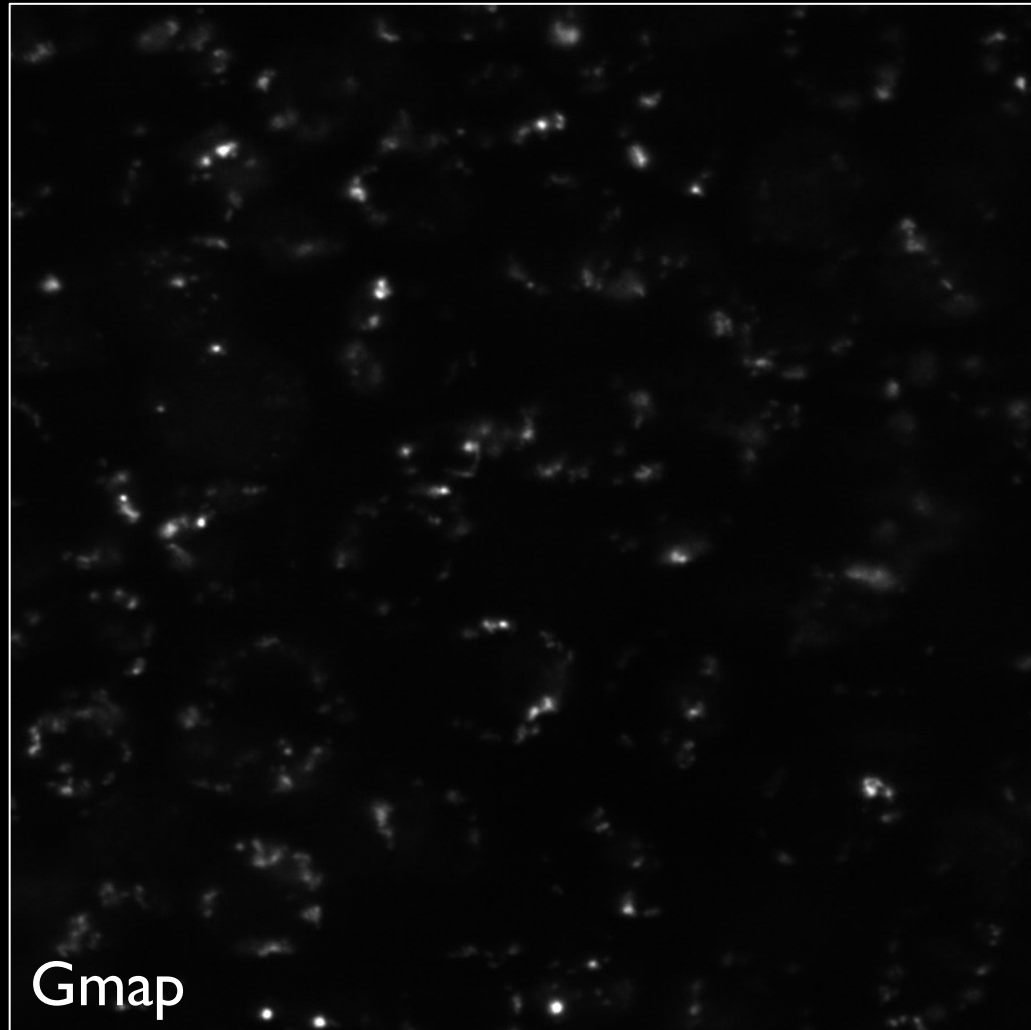


Endoplasmic Reticulum

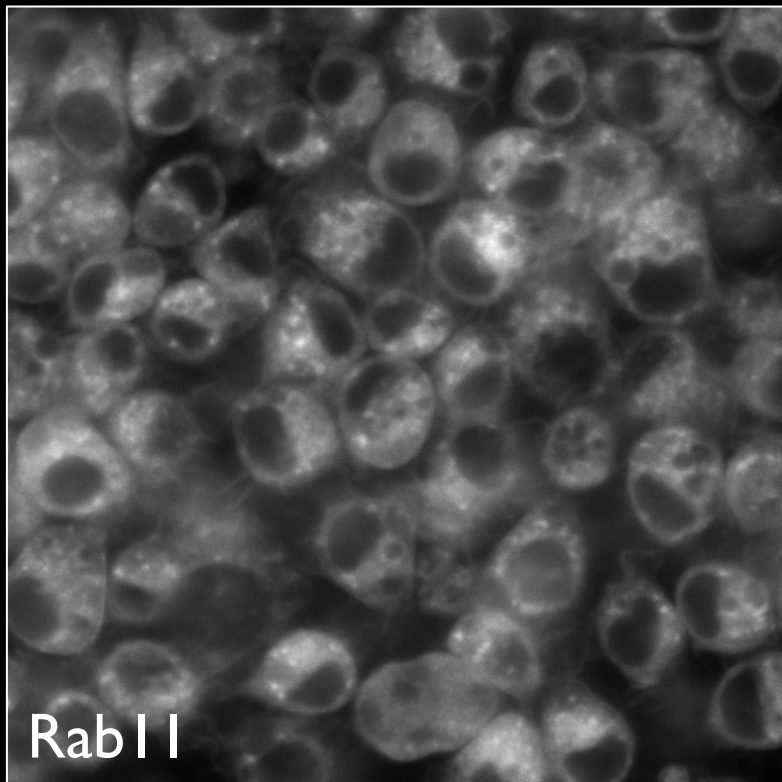


GFP live images

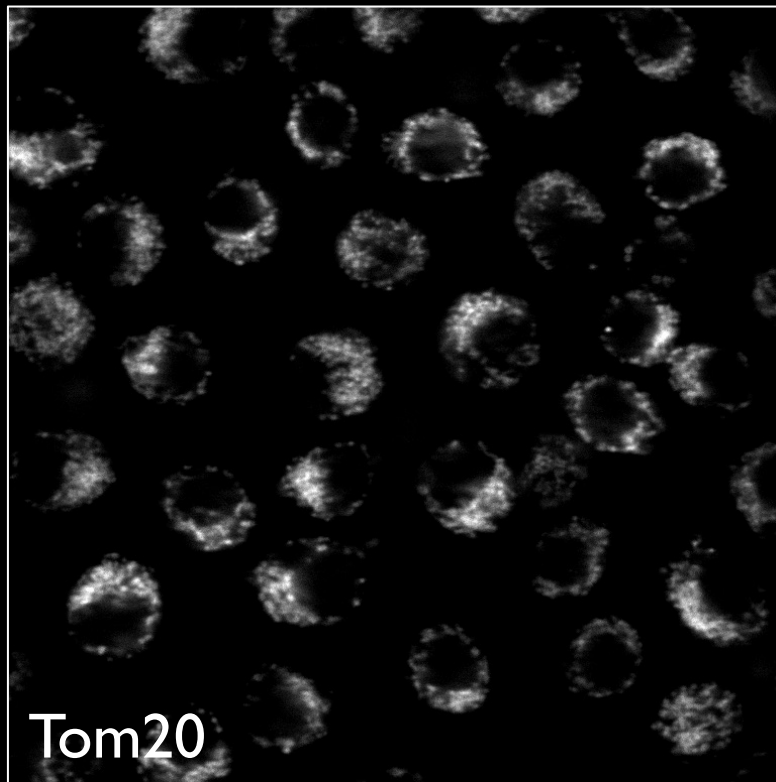
Golgi



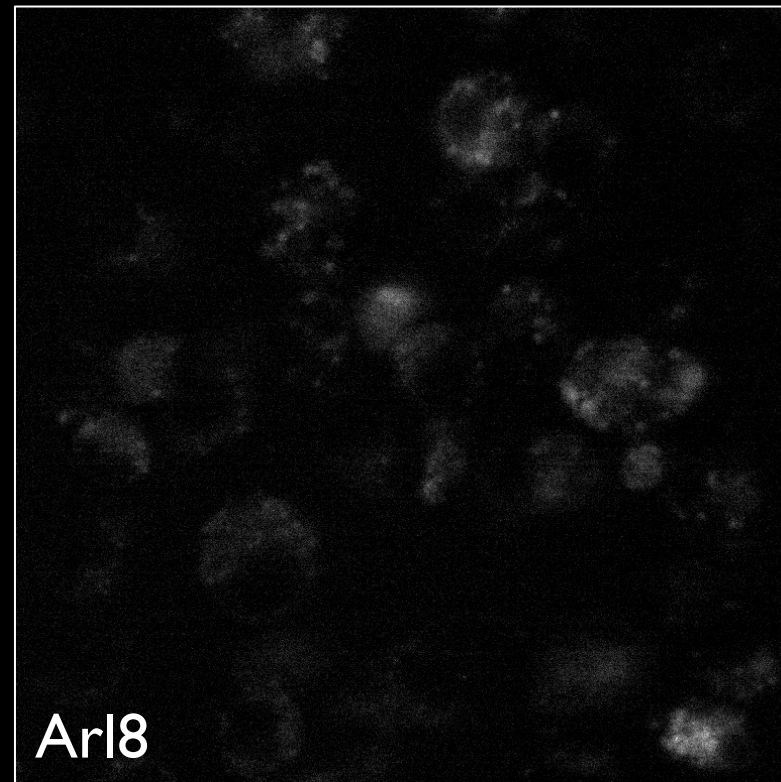
GFP live images



Endosomes

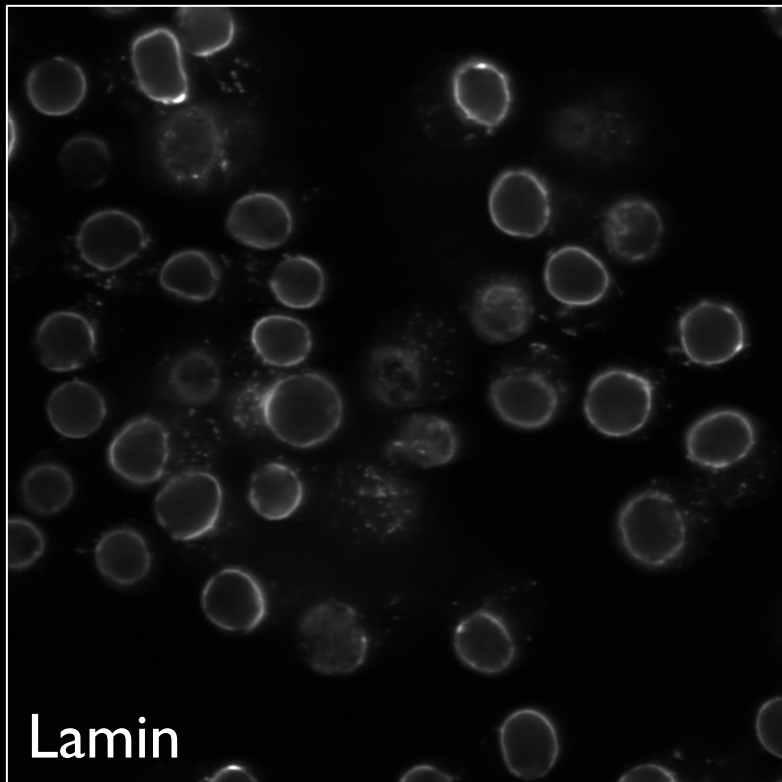


Mitochondria



Lysosomes

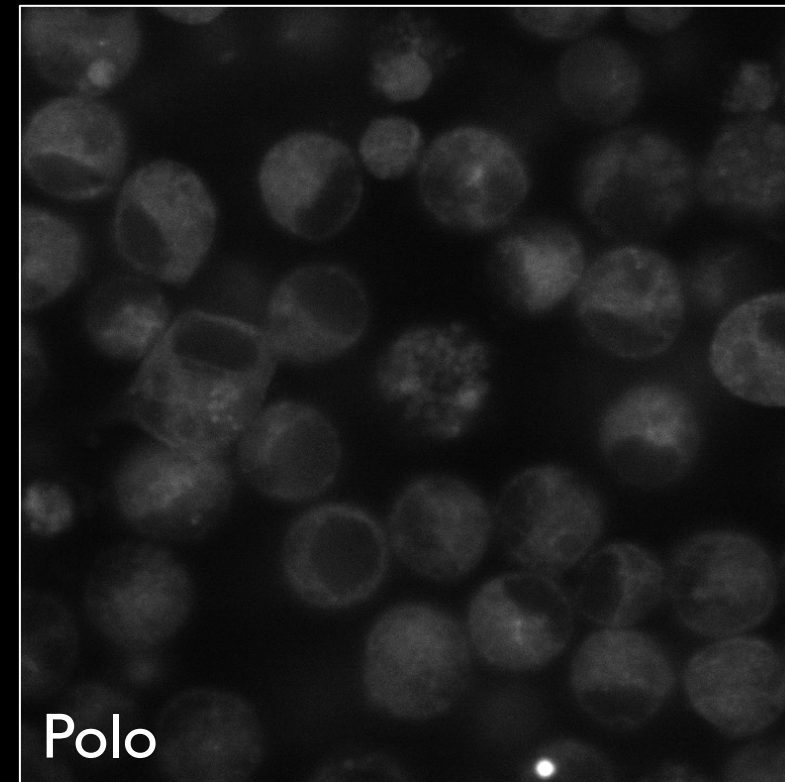
GFP live images



Nuclear Envelope

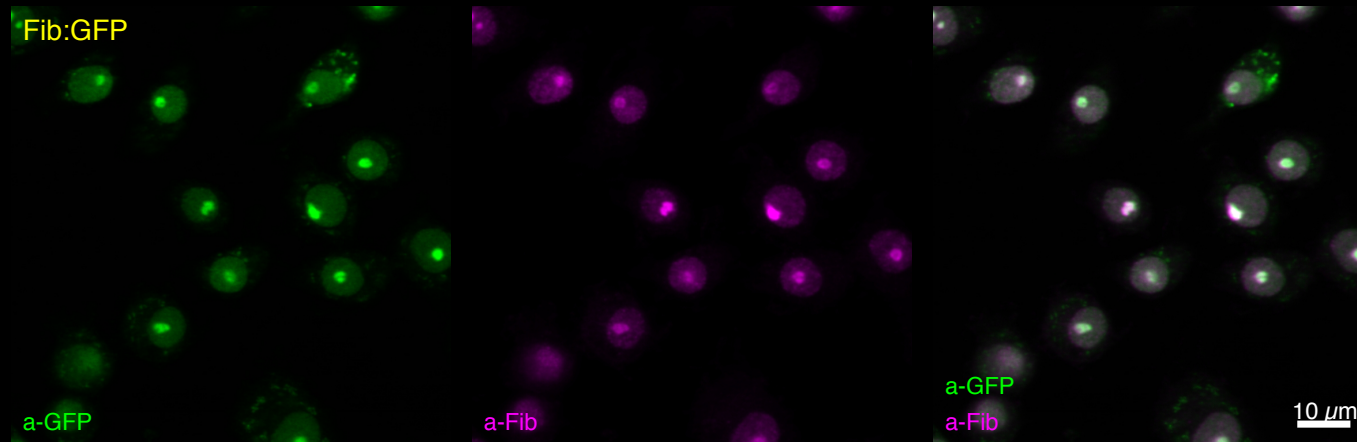
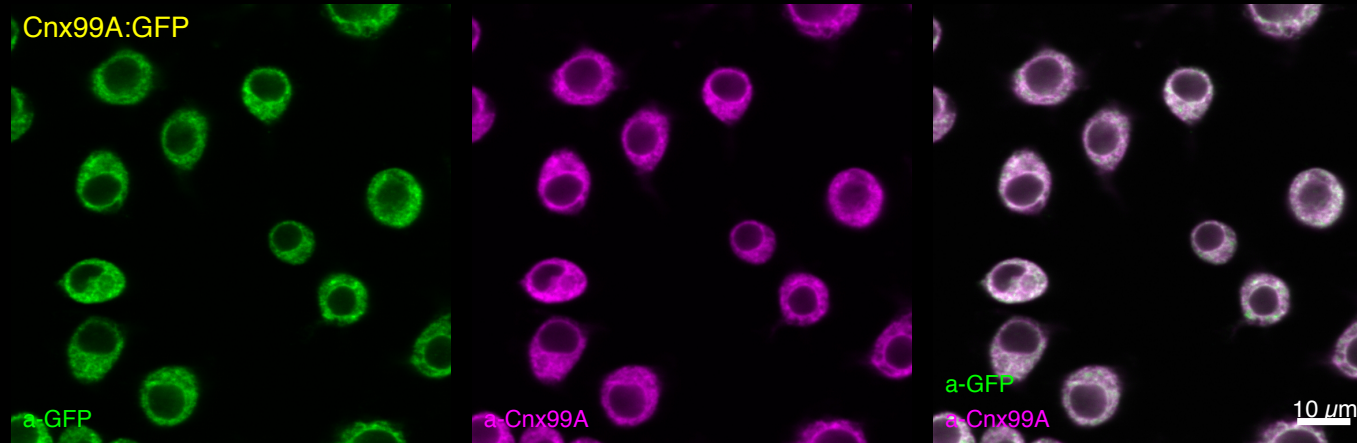


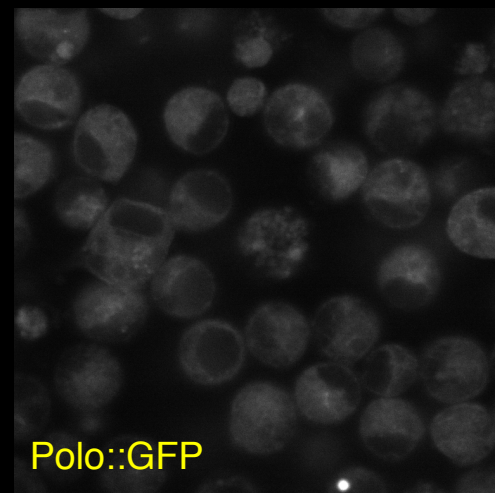
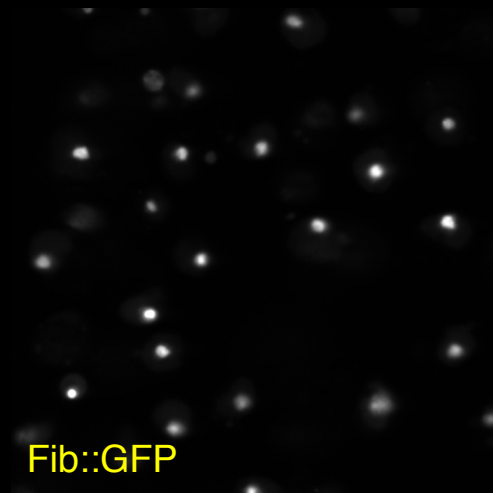
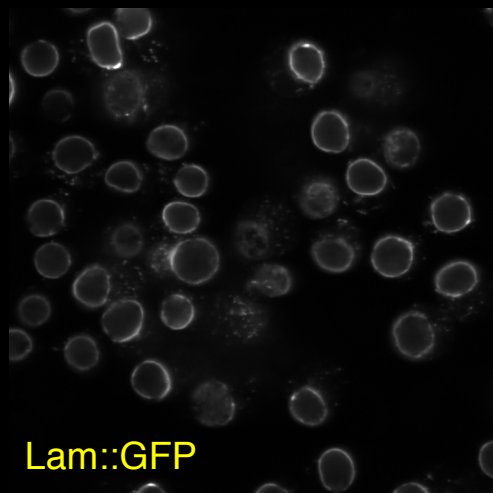
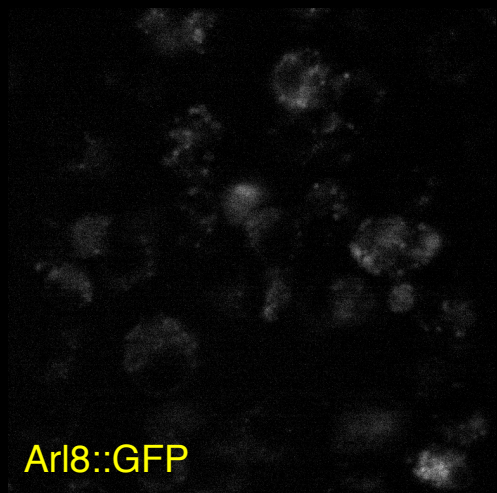
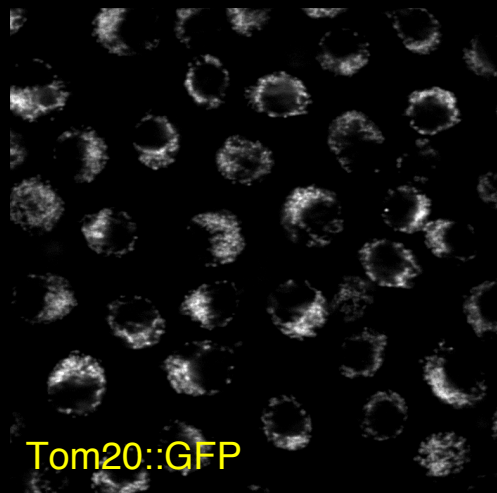
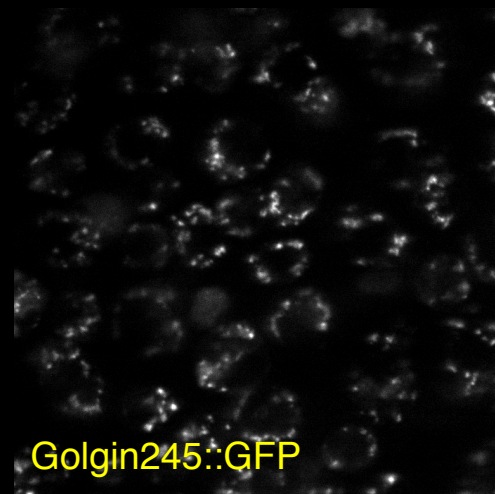
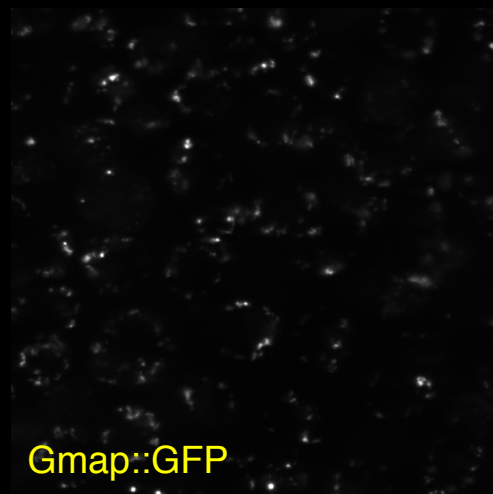
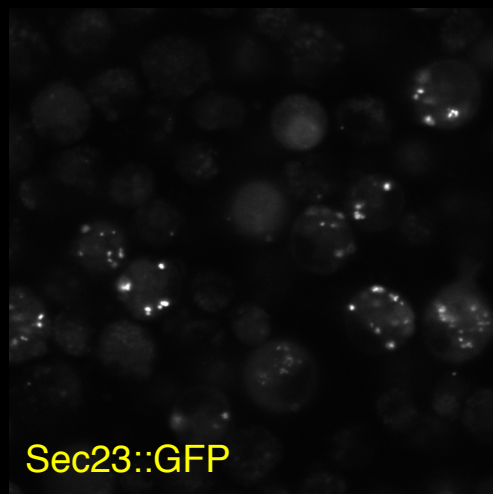
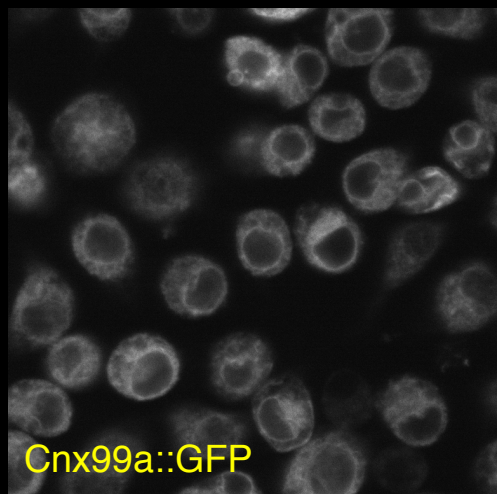
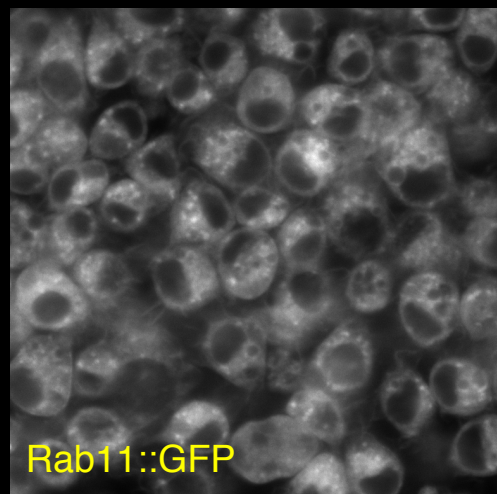
Nucleolus



Kinetochore

Fixed & Co-Stained





CRISPaint 'armless' donor knock-in

CSH Cold Spring Harbor Laboratory **bioRxiv** THE PREPRINT SERVER FOR BIOLOGY HOME | ABOUT

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New Results Comment on this paper

Gene knock-ins in *Drosophila* using homology-independent insertion of universal donor plasmids

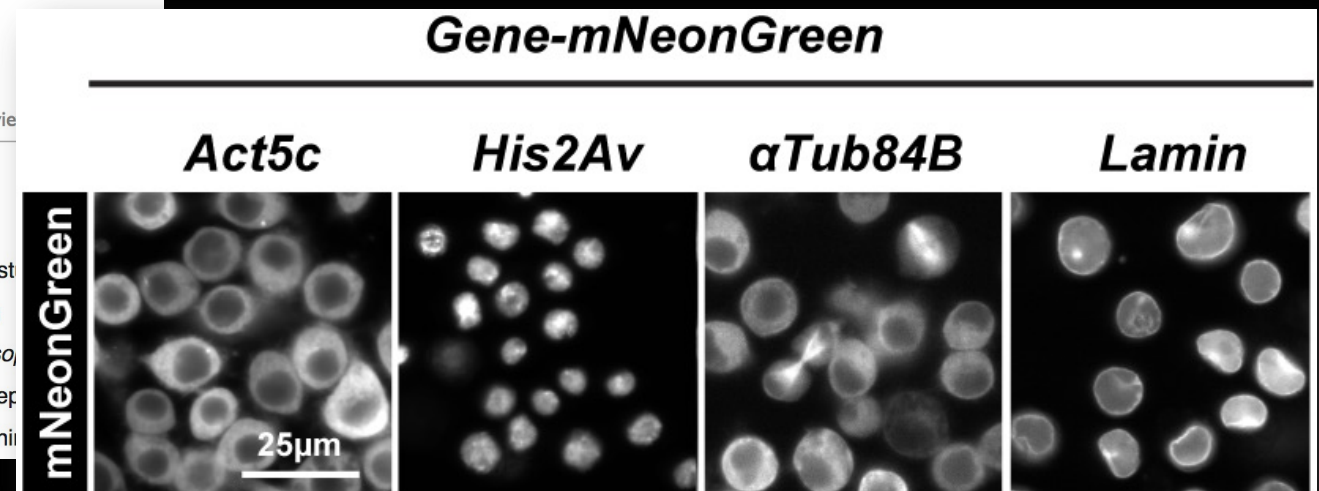
Justin A. Bosch, Ryan Colbeth, Jonathan Zirin, Norbert Perrimon
doi: <https://doi.org/10.1101/639484>
This article is a preprint and has not been peer-reviewed [what does this mean?].

Abstract Full Text Info/History Metrics Preview

Abstract

Site-specific insertion of DNA into endogenous genes (knock-in) is a powerful method to study gene function. However, traditional methods for knock-in require laborious cloning of long homology arms for homology-directed repair. Here, we report a simplified method in *Drosophila melanogaster* to insert large DNA elements into any gene using homology-independent repair. This method, known as CRISPaint, employs CRISPR-Cas9 and non-homologous end joining.

- C-terminal fusions
- Universal donor
- Antibiotic selection
- mNeonGreen



Bosch et al. 2019

Based on Schmid-Burgk et al. 2016 *Nature Communications*



ssDNA artificial exon



CRISPaint



Plasmid-based donor

- Insert anywhere

Limitation:
Difficult to build

ssDNA, artificial exon

- Easy to build
- Efficient

Limitation:
Gene has an intron

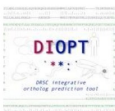
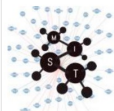
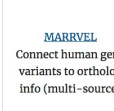
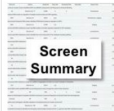
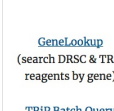
CRISPaint

- Pre-Made Donors
- Antibiotic selection

Limitation:
C-terminal tag only

Also at the DRSC/TRiP-FGR

- RNAi arrayed-format screens
- CRISPR pooled-format screens
- *in vivo* CRISPR
- TRiP-KO sgRNA fly stocks
- TRiP-OE sgRNA fly stocks
- *nominate!*
- New online tools
 - iProteinDB—phosphorylation
 - BioLitMine—literature mining tool

Multi-Species				
 DIOPT DRSC integrative ortholog prediction tool	 Gene2Function	 BioLitMine	 MIST	 MARRVEL
DIOPT ortholog search 10 species, 18 algorithms [Demo Video]	Gene2Function orthologs & gene info summaries (orthologs, GO, publications, & more)	BioLitMine literature mining tool (genes, pathways, people, MeSH terms) [Demo Video]	MIST protein-protein & genetic interactions (multi-source) [Demo Video]	MARRVEL Connect human gene variants to ortholog info (multi-source)
				DIOPT-DIST Connect disease genes to ortholog info or vice versa (OMIM & GWAS)
Fly CRISPR		Fly PTMs		
 fly sgRNA database/LIMS	 Find CRISPRs	 CRIMIC CRISPR MIMIC Gene Trap	 RSVP Plus	 YTS iProteinDB
TRiP sgRNA LIMS nominate or track TRiP-KO & -OE fly stock production	Find CRISPRs fly sgRNA design with genome view	CRIMIC CRISPR MIMIC Gene Trap fly sgRNA design with genome view	RSVP Plus in vivo CRISPR & RNAi phenotype data	YTS iProteinDB fly post-translational modifications (multi-source)
Fly RNAi		Screen Summary		
 UP-TORR	 SnapDragon	 SVP Plus	 Screen Summary	 GeneLookup
UP-TORR cell and in vivo RNAi reagent search	SnapDragon design dsRNAs for fly cell RNAi	SVP Plus CRISPR & RNAi	Screen Summary browse DRSC cell RNAi	GeneLookup (search DRSC & TRiP reagents by gene)
TRiP Batch Query (make a TRiP fly stock list from a gene list)				
More fly resources				
 DGET Drosophila Gene Expression Tool	 GLAD Gene List Annotation for Drosophila	 FlyPrimerBank	 FlyPrimerBank	
DGET mine bulk RNAseq data for fly	GLAD view grouped gene lists for fly	FlyPrimerBank find qPCR primers for fly studies	FlyPrimerBank find qPCR primers for fly studies	
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at Baylor:

Oguz Kanca

Hugo Bellen

Funding:

NIH ORIP R24 OD019847

NIH NIGMS R01 GM067761

*See next page for info on
requesting cells and protocols*

DRSC/TRiP Staff and Perrimon lab!



@DRSC_TRiP

<https://fgr.hms.harvard.edu>



Cell Line Requests & Relevant Publications

- **How to request cells:** the DRSC (Harvard Med School) shared the cell lines with the DGRC (Bloomington) for distribution to the community. See this URL on our DRSC site to get started requesting the cells from the DGRC. <https://fgr.hms.harvard.edu/crispr-modified-cell-lines>
- **Research publication:** Kanca O, Zirin J, Garcia-Marques J, Knight SM, Yang-Zhou D, Amador G, Chung H, Zuo Z, Ma L, He Y, Lin VWW, Fang Y, Ge M, Yamamoto S, Schulze KL, Hu Y, Spradling AC, Mohr SE, Perrimon N, Bellen HJ. An efficient CRISPR-based strategy to insert small and large fragments of DNA using short homology arms. Elife. 2019 Nov 1;8:e51539. doi: 10.7554/eLife.51539. PMID: 31674908; PMCID: [PMC6855806](#).
- **Protocol publication:** Bosch JA, Knight S, Kanca O, Zirin J, Yang-Zhou D, Hu Y, Rodiger J, Amador G, Bellen HJ, Perrimon N, Mohr SE. Use of the CRISPR-Cas9 System in Drosophila Cultured Cells to Introduce Fluorescent Tags into Endogenous Genes. Curr Protoc Mol Biol. 2020 Mar;130(1):e112. doi: 10.1002/cpmb.112. PMID: 31869524; PMCID: [PMC7213786](#).