GFP tagging in *Drosophila* cultured cells

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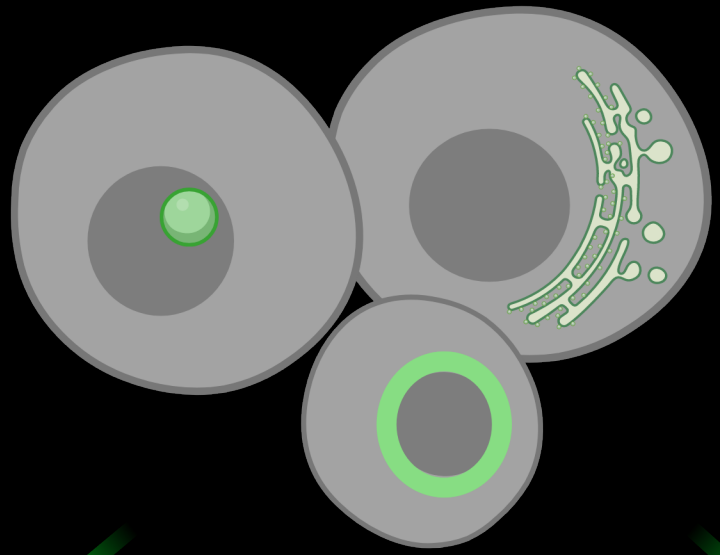
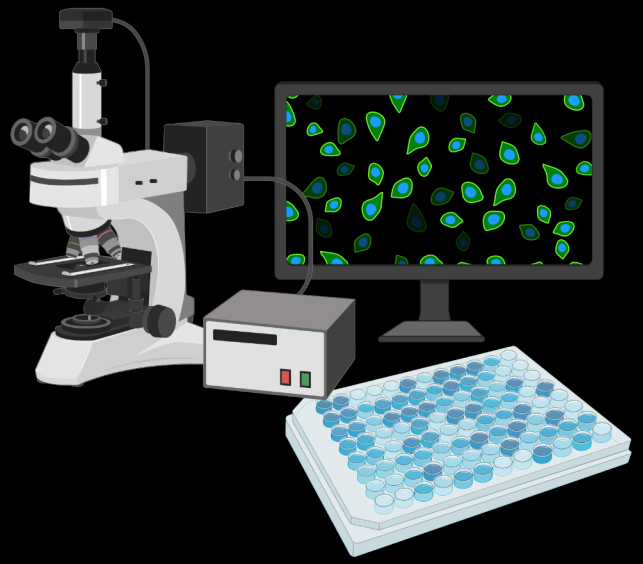
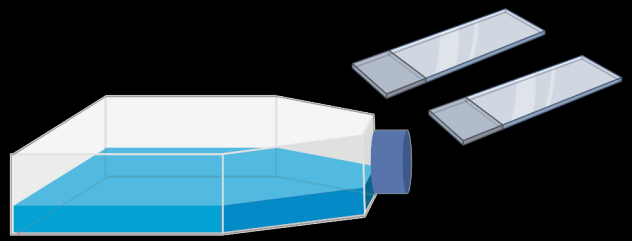
Why fluorescent protein tags?



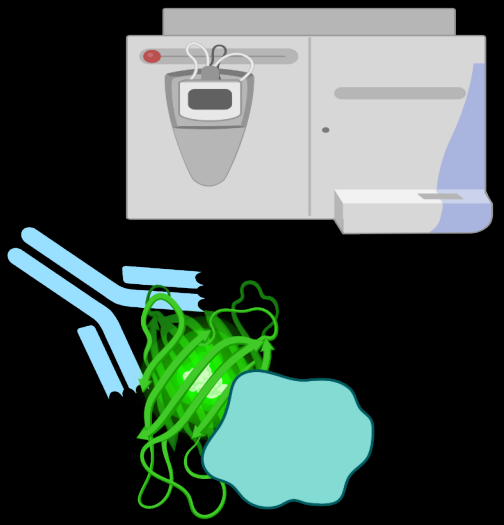
- Sub-cellular distribution
- Endogenous levels



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



Detailed Cell Biological Studies
(live cell, time lapse)

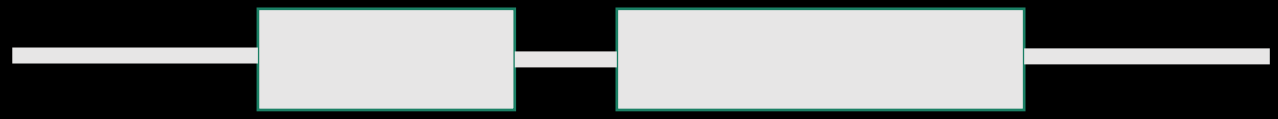


Proteomics Assays
(anti-GFP reagents)

CRISPR knock-in approaches

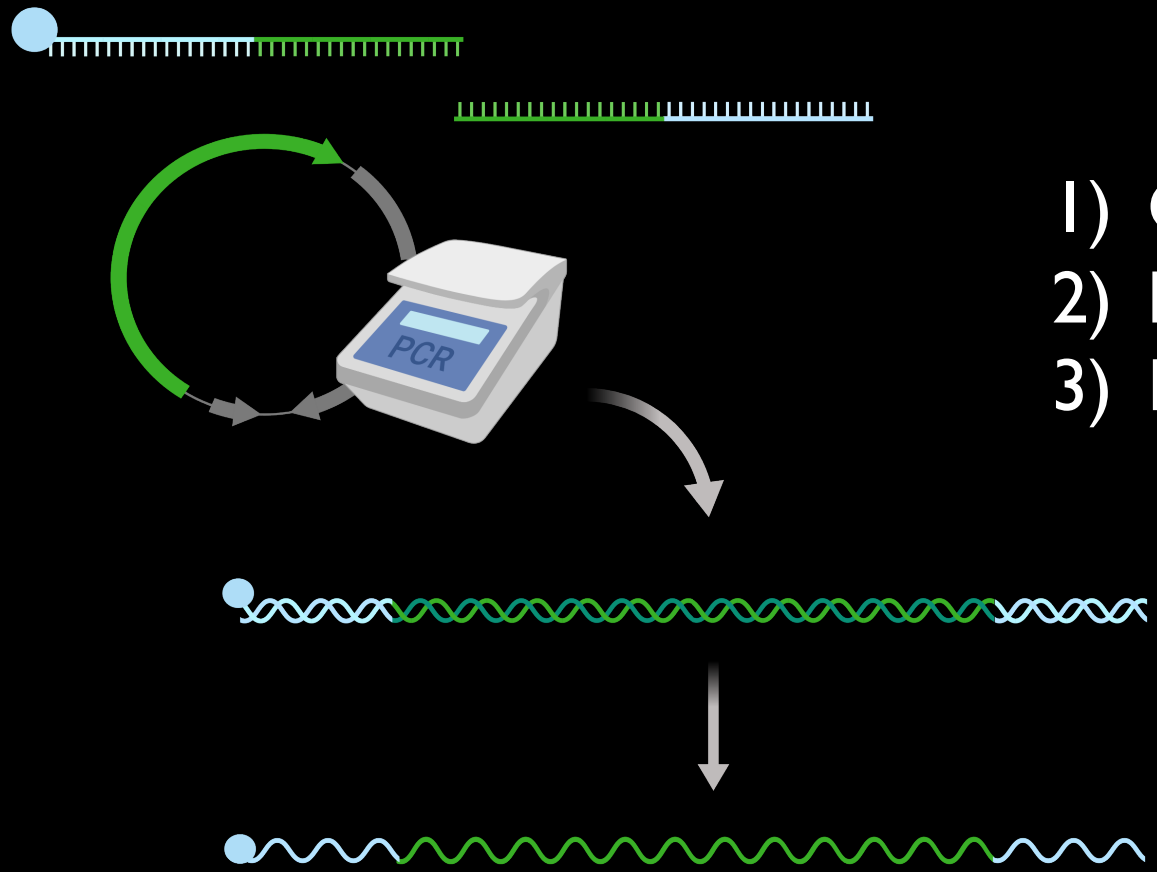
- plasmid-based donors, long homology arms
- single-stranded DNA donors, short homology arms
- ‘armless’ donors (CRISPaint method)

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



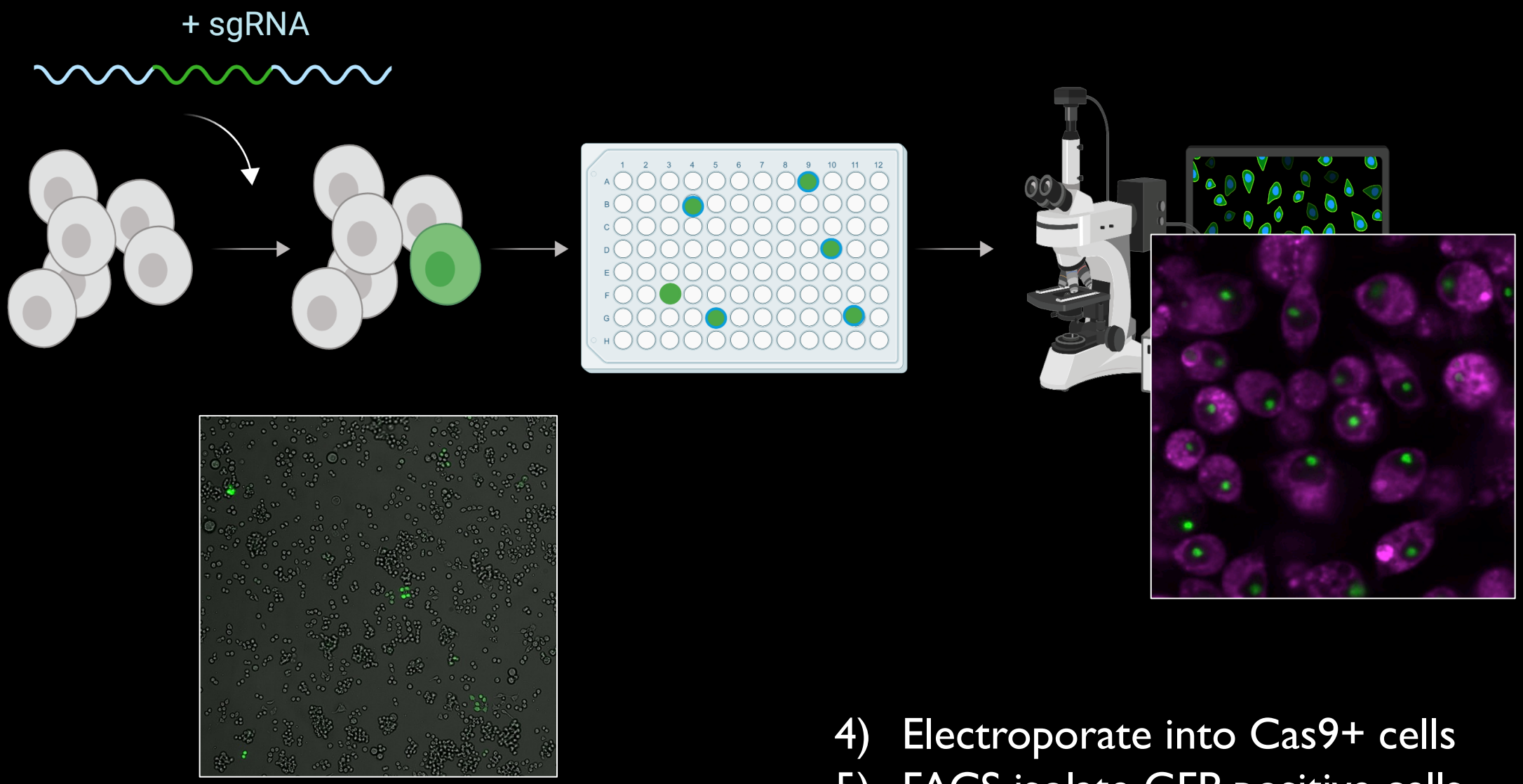
ssDNA artificial exon

ssDNA synthesis

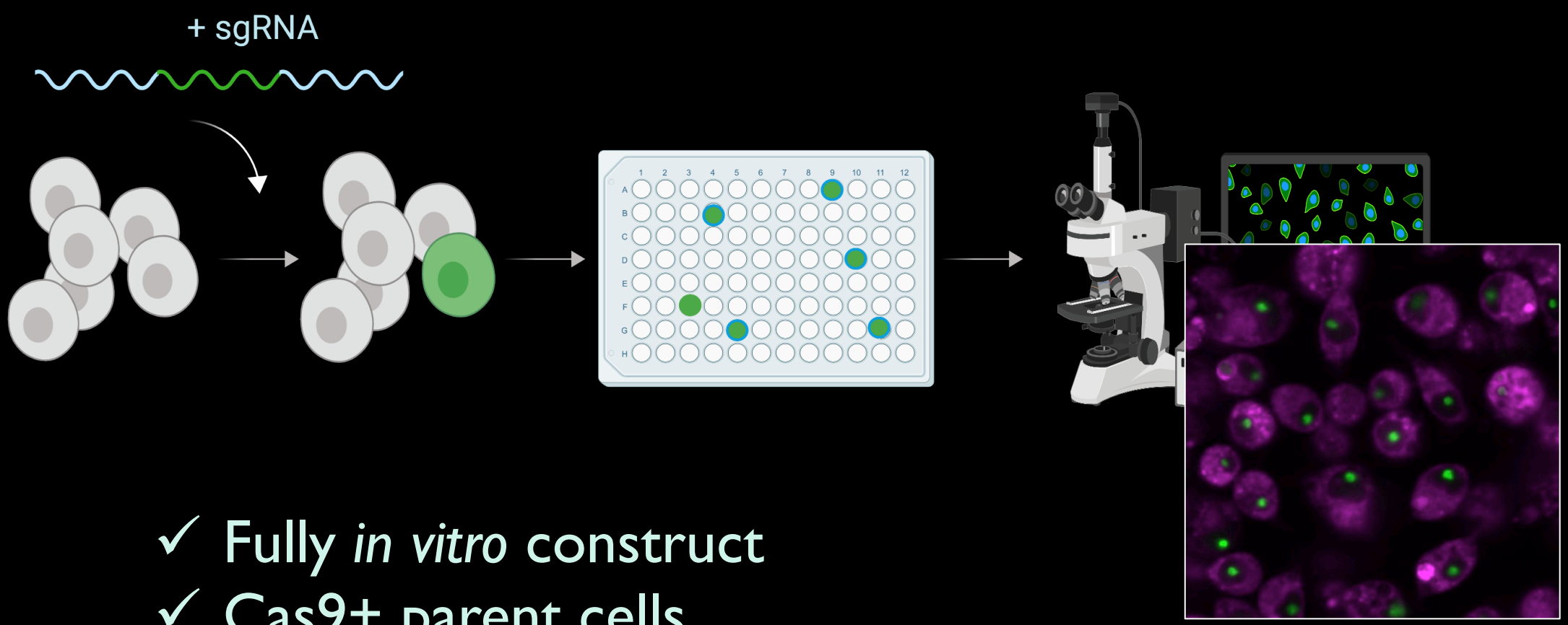


1) Oligos with short homology arms
2) PCR amplify GFP template
3) Digest to make ssDNA

Collaborators: O. Kanca & H. Bellen



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



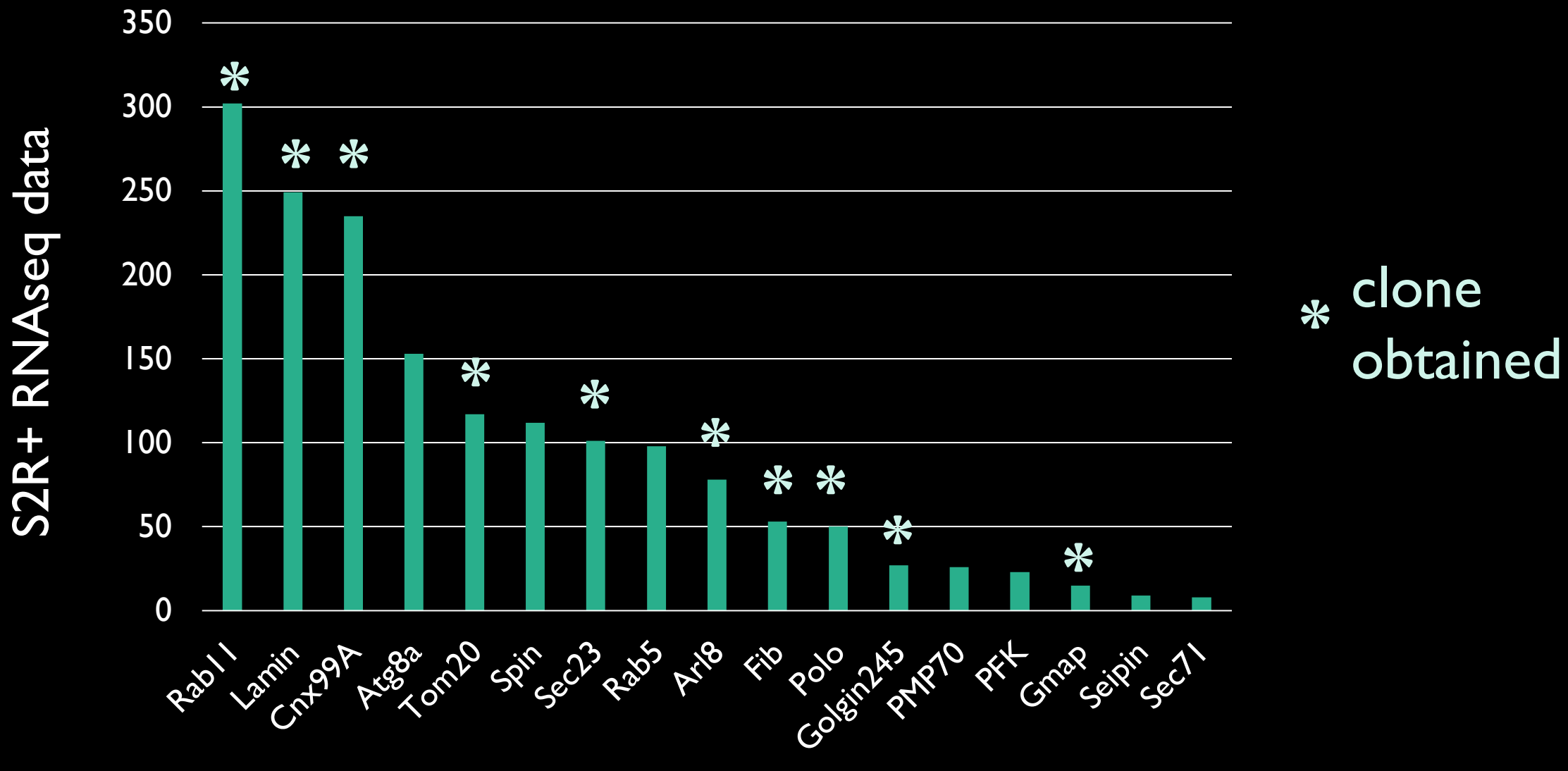
- ✓ Fully *in vitro* construct
- ✓ Cas9+ parent cells
- ✓ Electroporation
- ✓ Conditioned Media

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

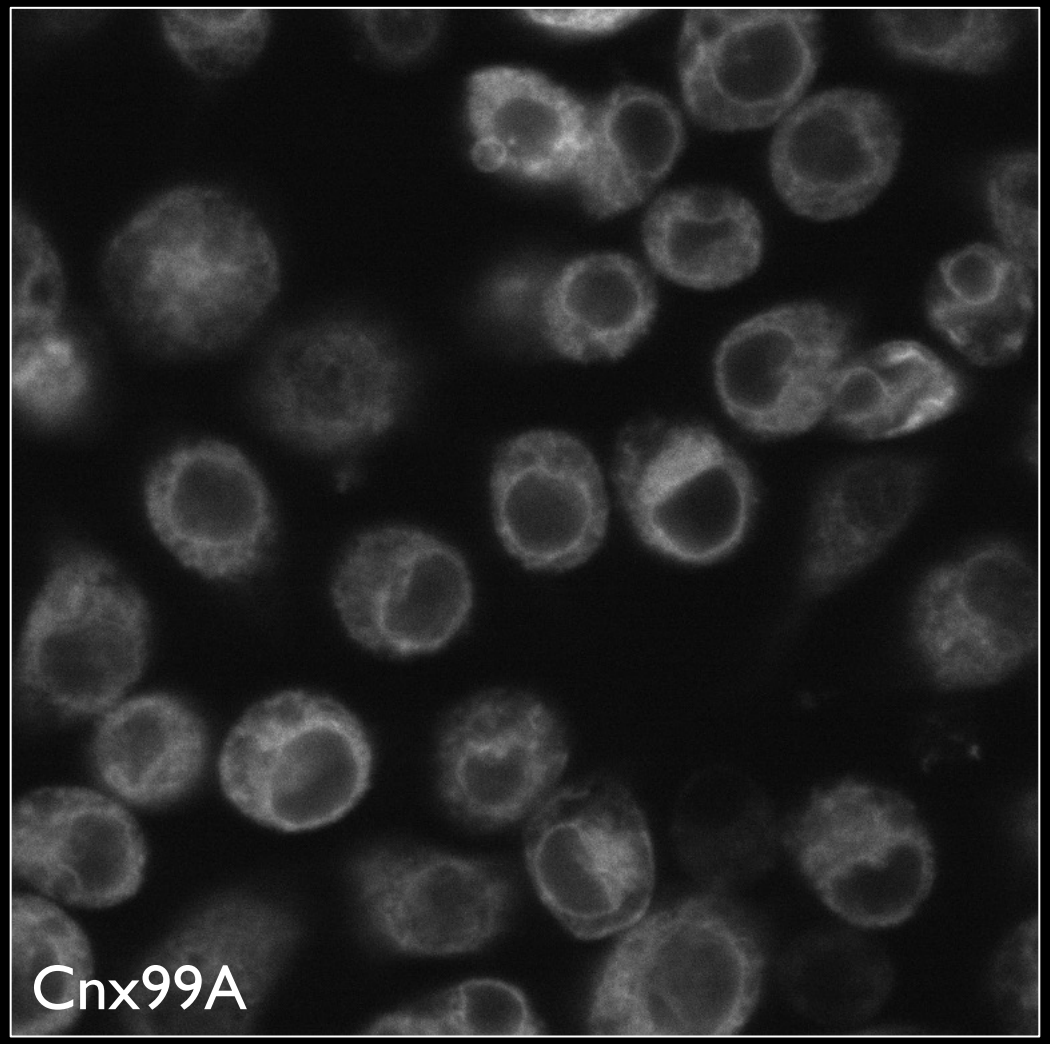
Community Access:
Drosophila Genome Resource Center (DGRC) cell line repository in Bloomington, IN

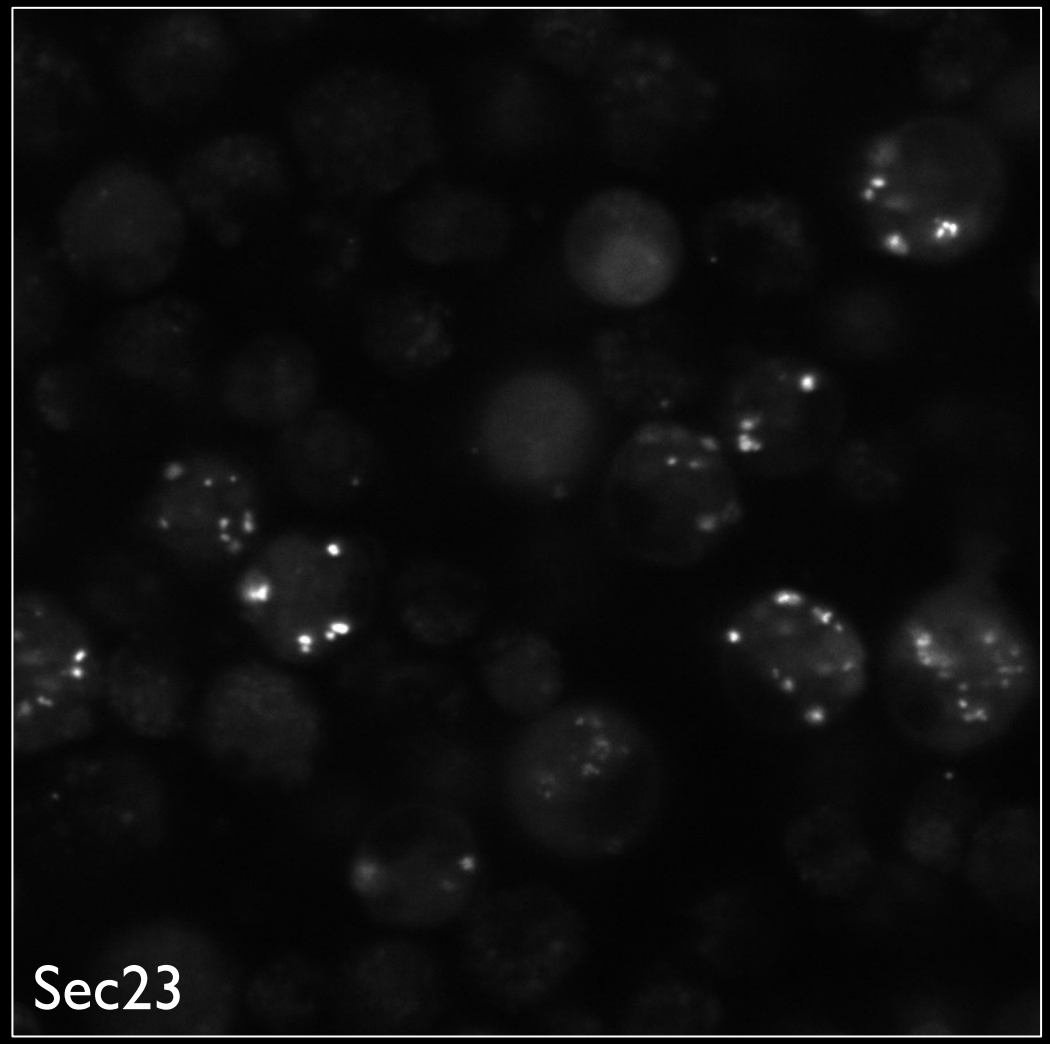
Expression vs. clone obtained



GFP live images

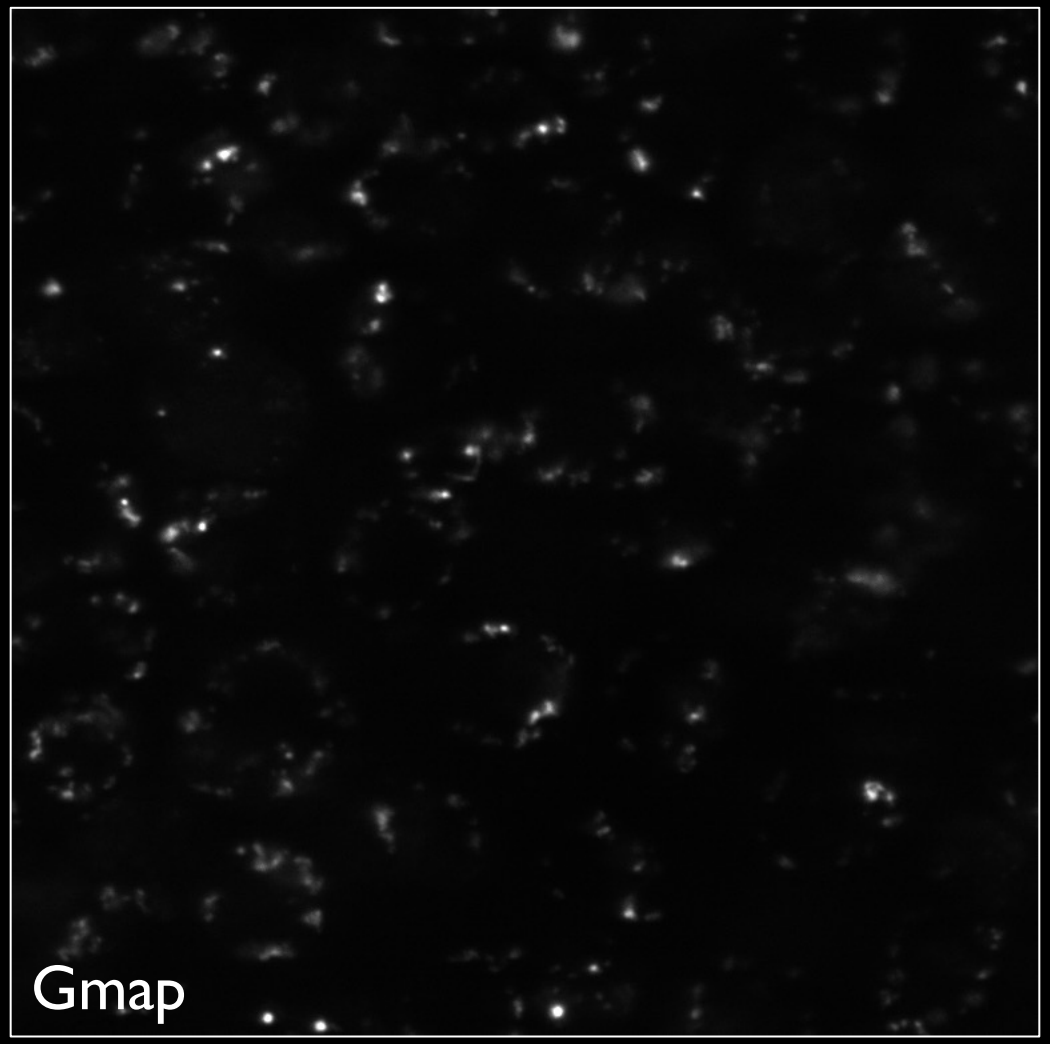
Endoplasmic Reticulum

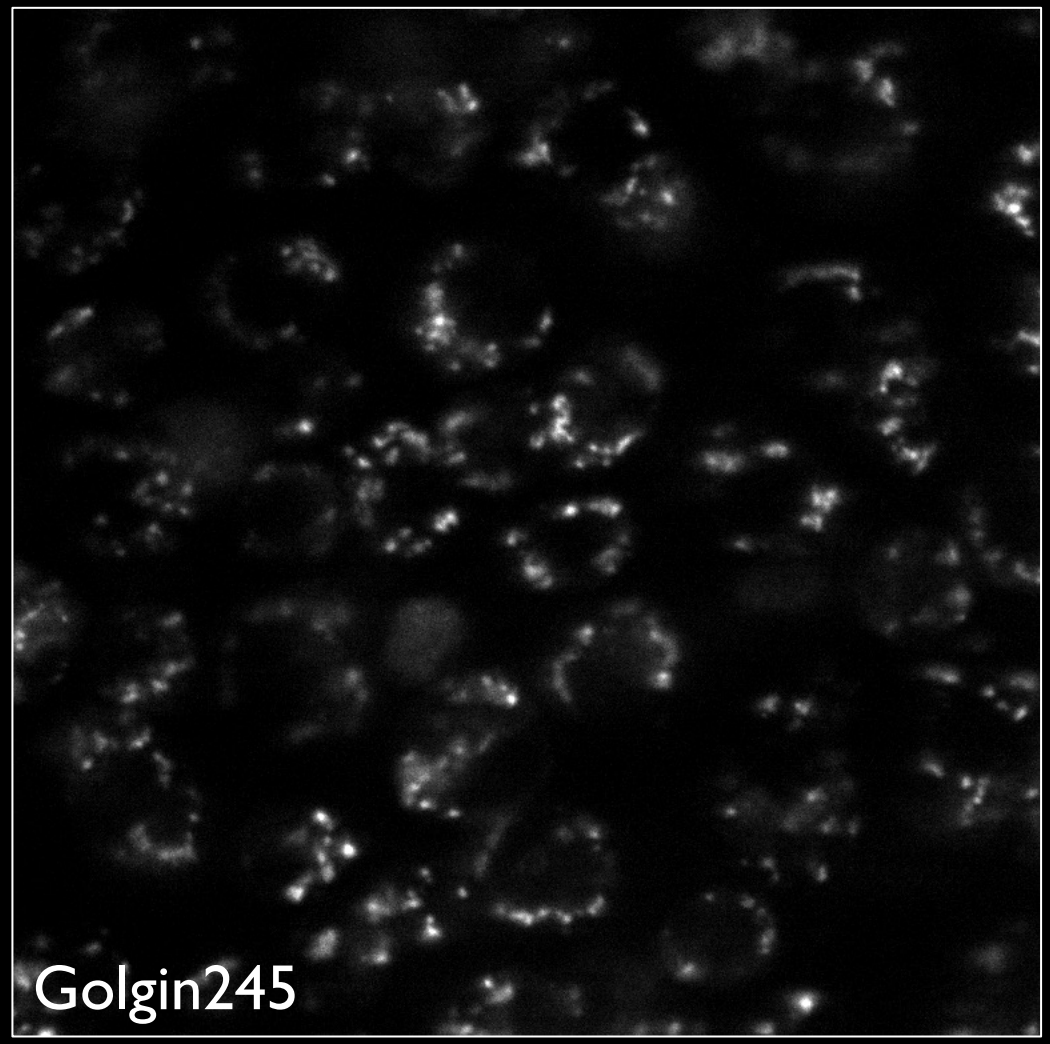




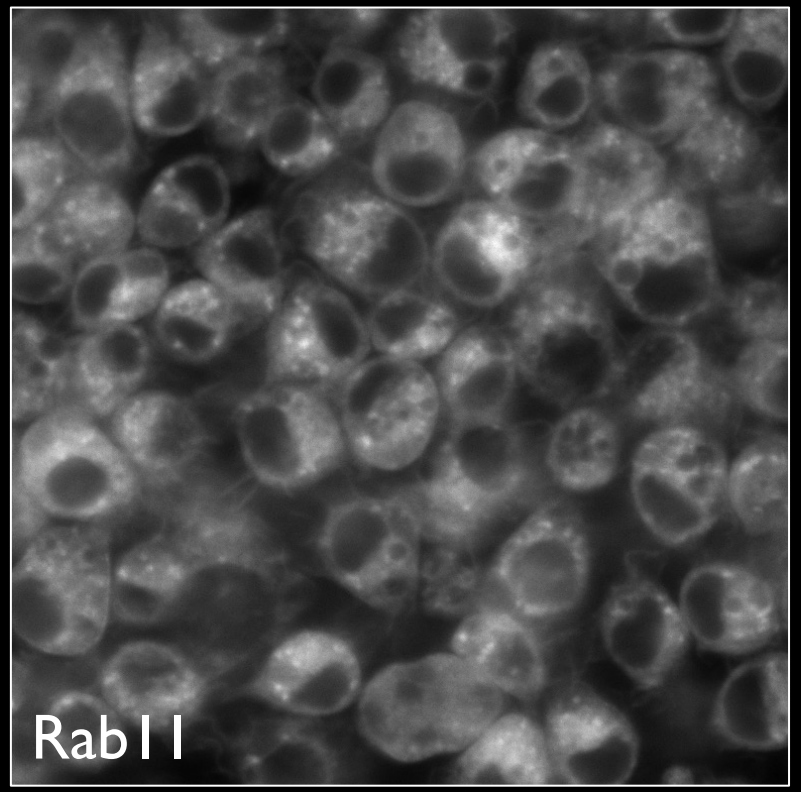
GFP live images

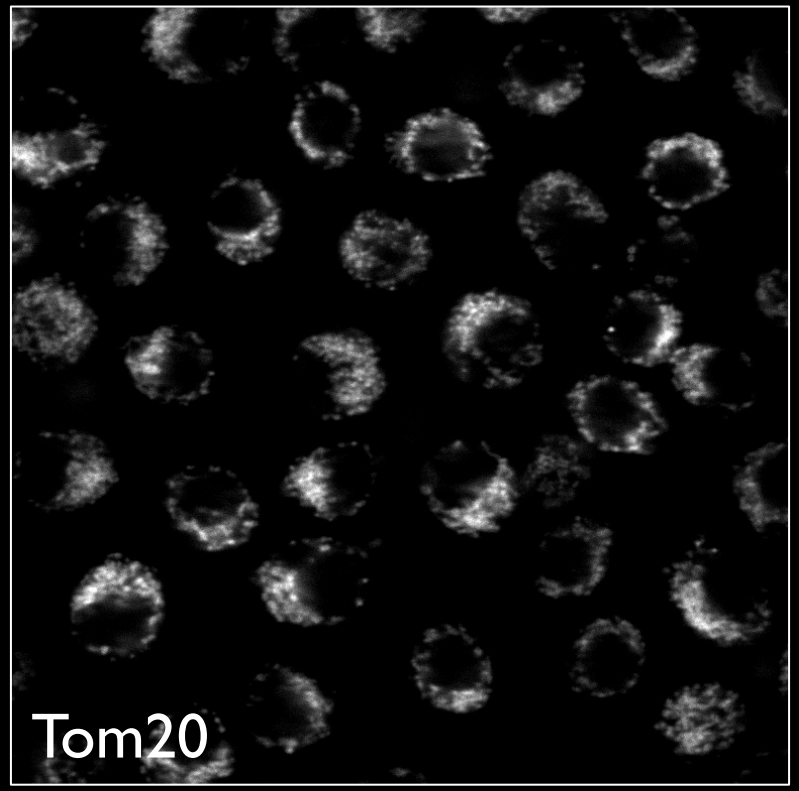
Golgi

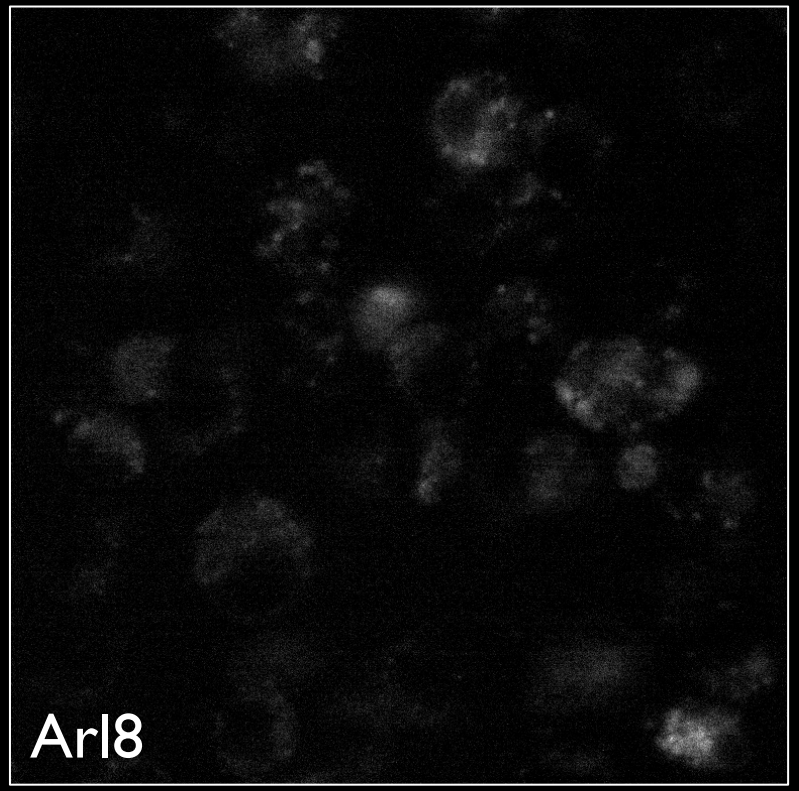




GFP live images





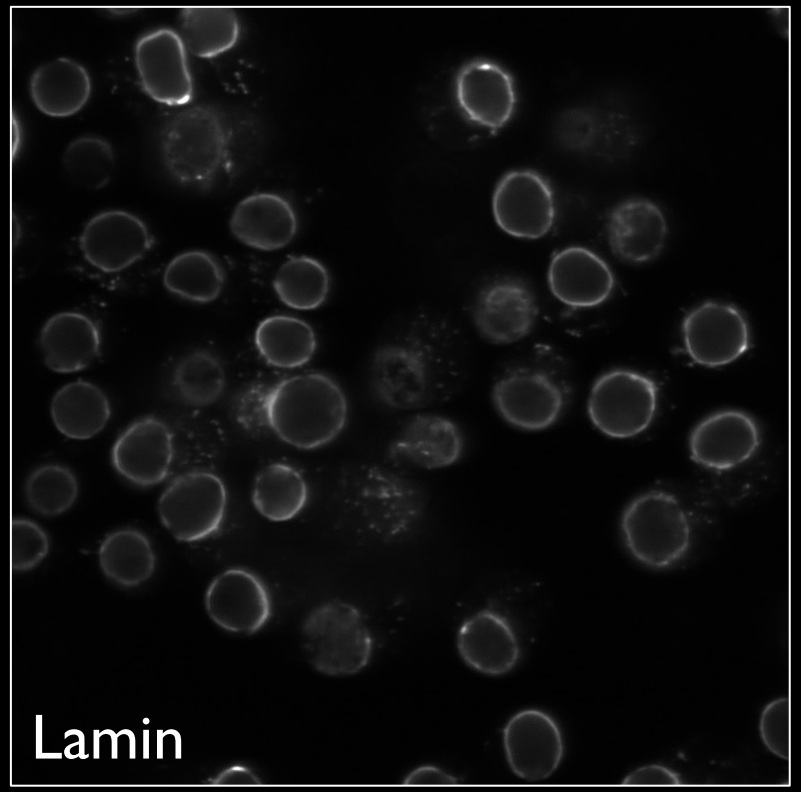


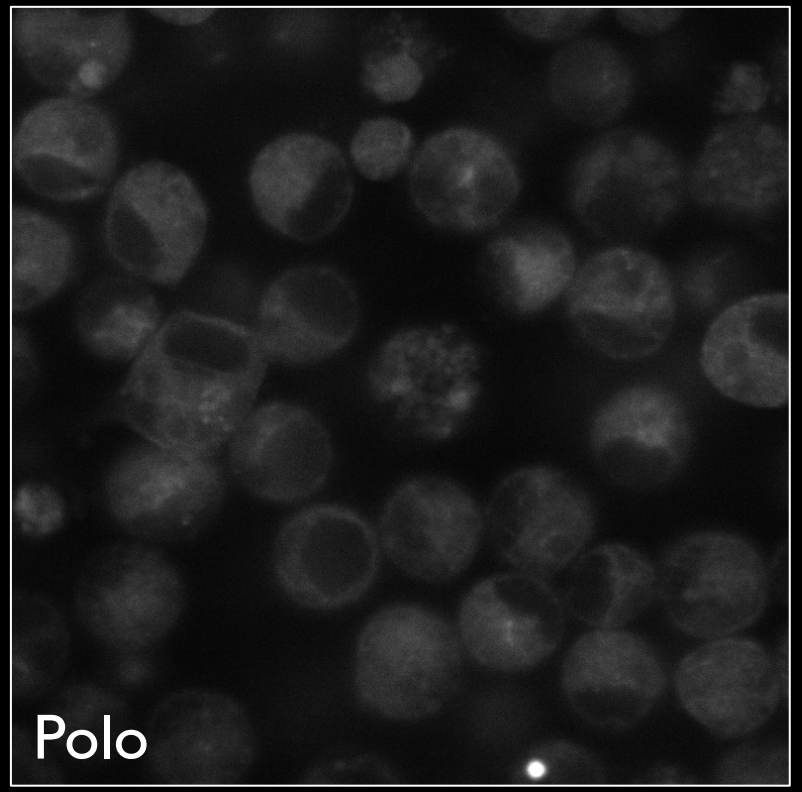
Endosomes

Mitochondria

Lysosomes

GFP live images



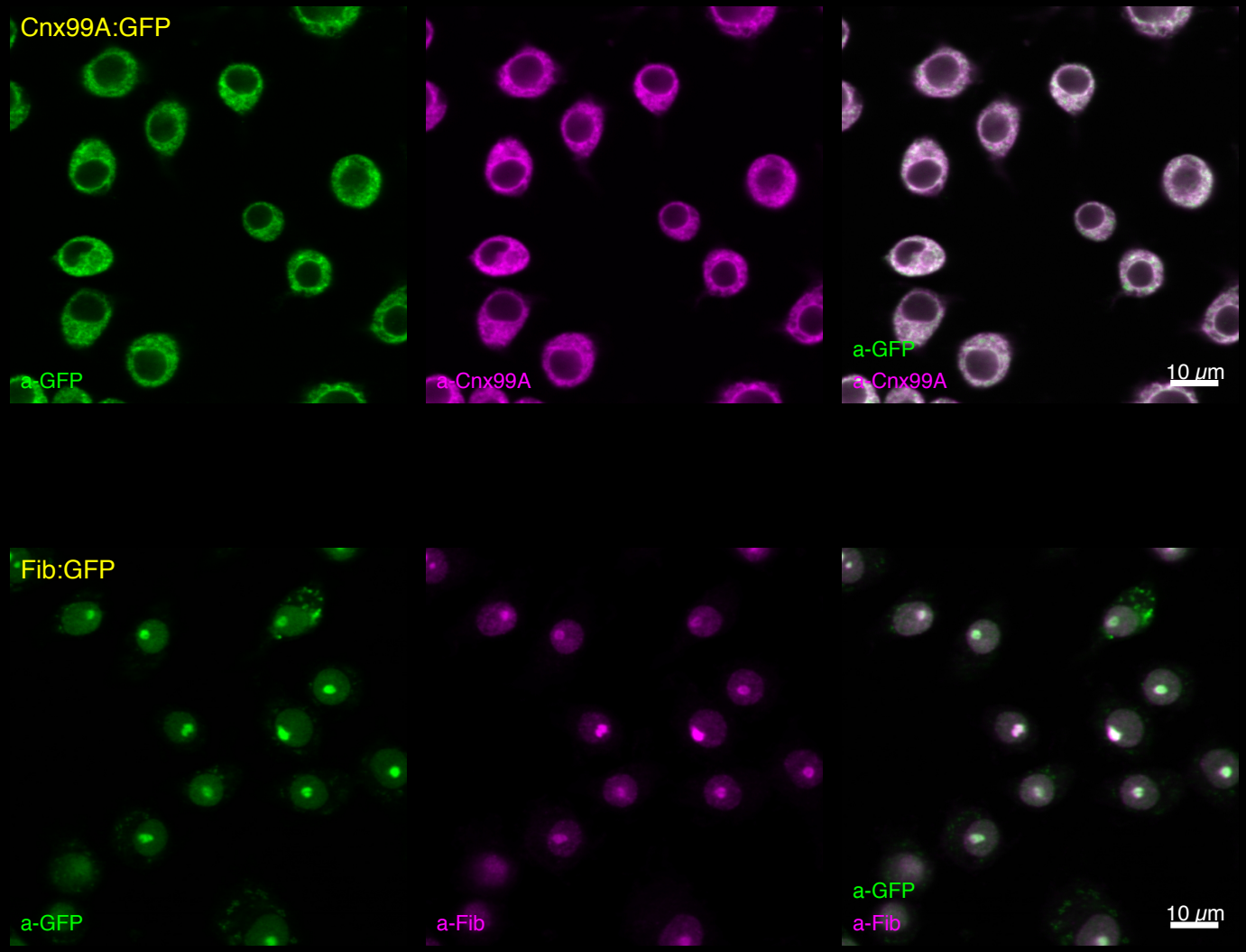


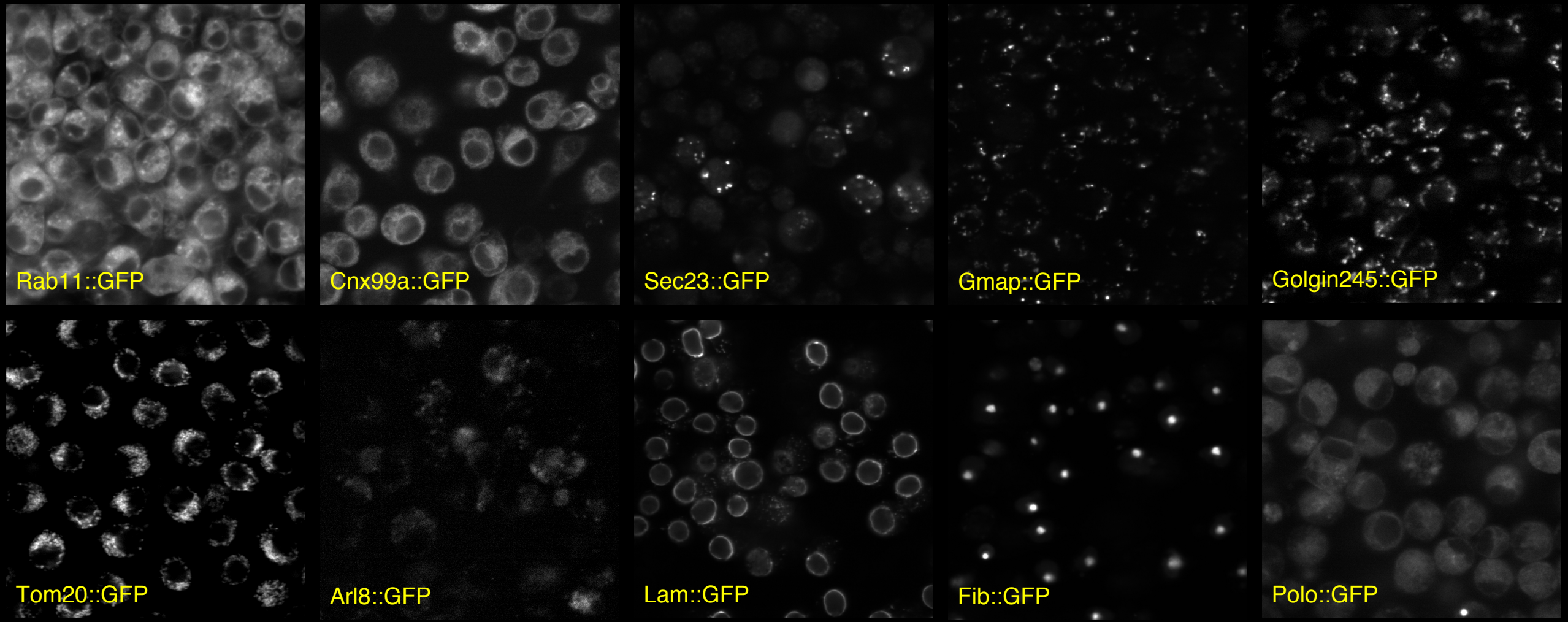
Nuclear Envelope

Nucleolus

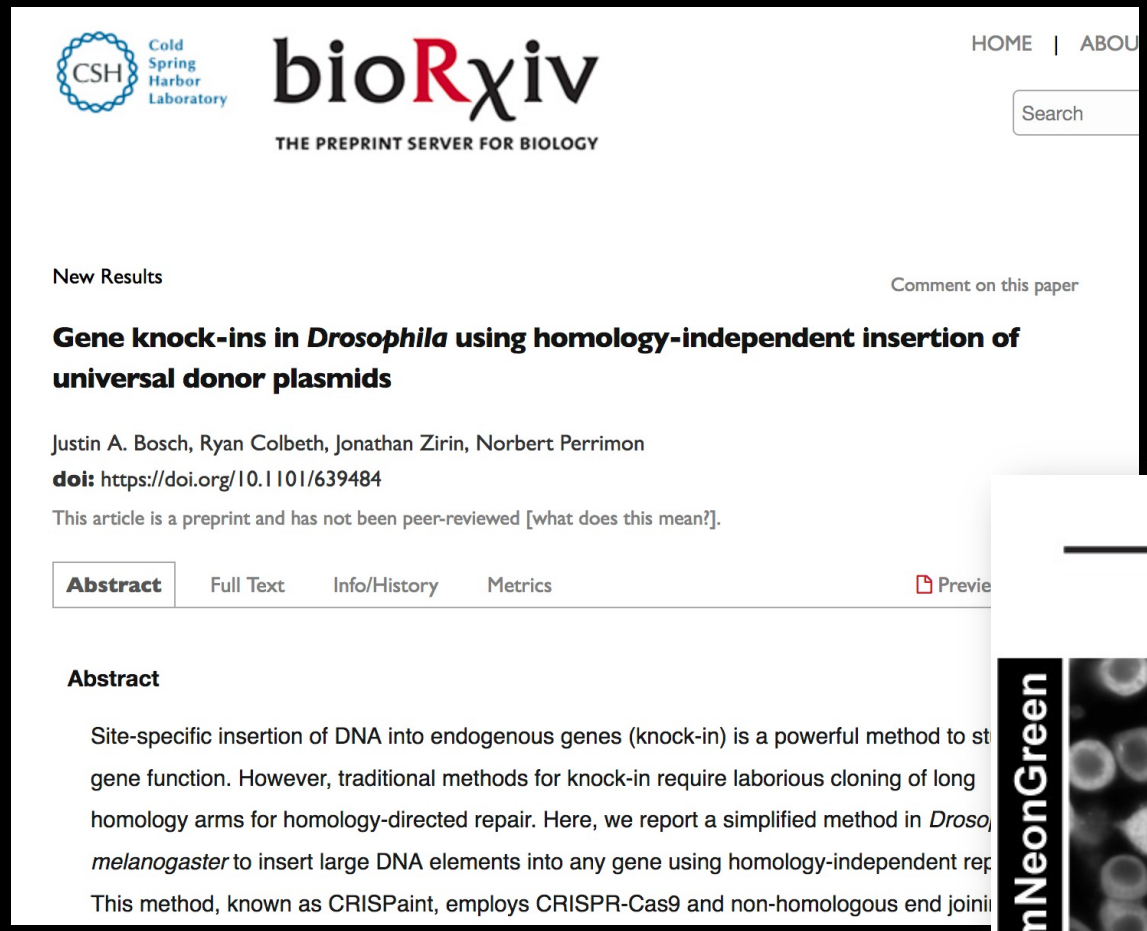
Kinetochore

Fixed & Co-Stained

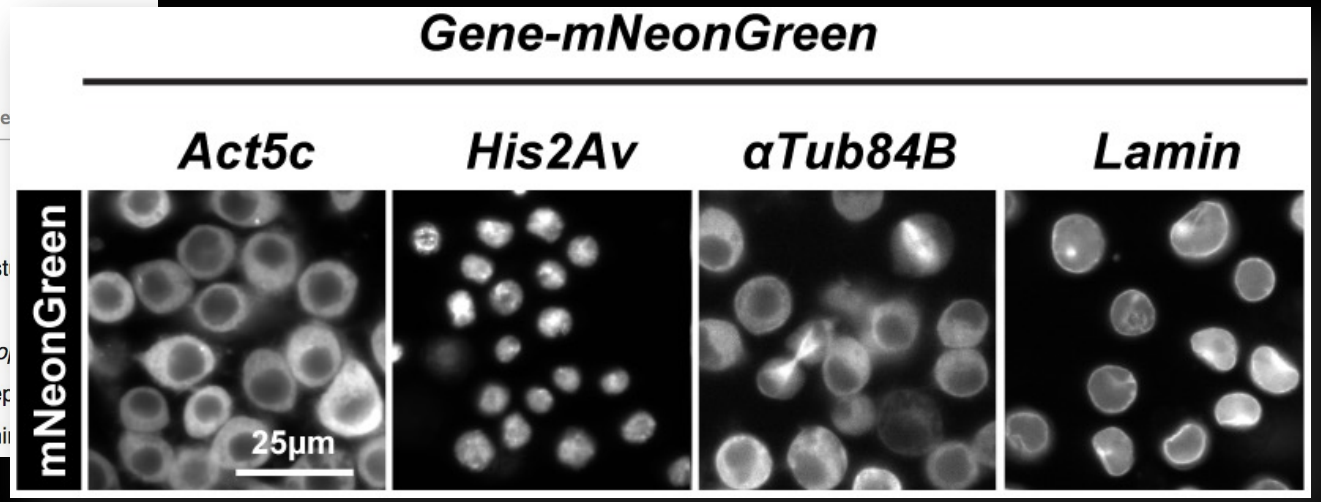



Rab11::GFP
Cnx99a::GFP
Sec23::GFP
Gmap::GFP
Golgin245::GFP
Tom20::GFP
Arl8::GFP
Lam::GFP
Fib::GFP
Polo::GFP

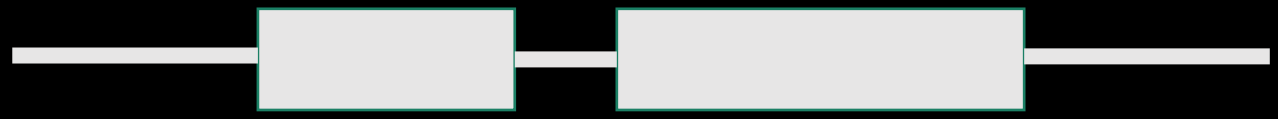
CRISPaint 'armless' donor knock-in



- 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

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at DRSC/TRiP:
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Ram Viswanatha
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Norbert Perrimon

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at Baylor:
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Hugo Bellen

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NIH NIGMS R01 GM067761

See next page for info on requesting cells and protocols

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https://fgr.hms.harvard.edu

NIH ORIP OFFICE OF RESEARCH INFRASTRUCTURE PROGRAMS
NIH NIGMS
hhmi Howard Hughes Medical Institute
DF/HCC Dana-Farber/Harvard Cancer Center
NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

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 WW, 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.