

Summer Intern 2023

Fumi Tanizawa

Harvey Mudd College (Class of 2025)

Dates: May 22, 2023 - Aug 22, 2023

Lab: Srinivasan Lab, TSRI

Mentors: Chung-Chih Liu & Anthony Perez

Research Focus & Project Outline

Research Focus:

The HLH-11 Feedback Loop: Bridging Fat Oxidation with Mitochondrial Stress Responses and Lifespan

Project Outline:

1. Cloning tissue-specific hlh-11 rescue construct.
2. hlh-11 CRISPR (Global Knockout).
3. Crossing of hlh-11 mutant with Phlh-11::hlh-11 GFP.
4. NeuroPAL x hlh-11 GFP Imaging.
5. Lifespan Experiment (with Esra).

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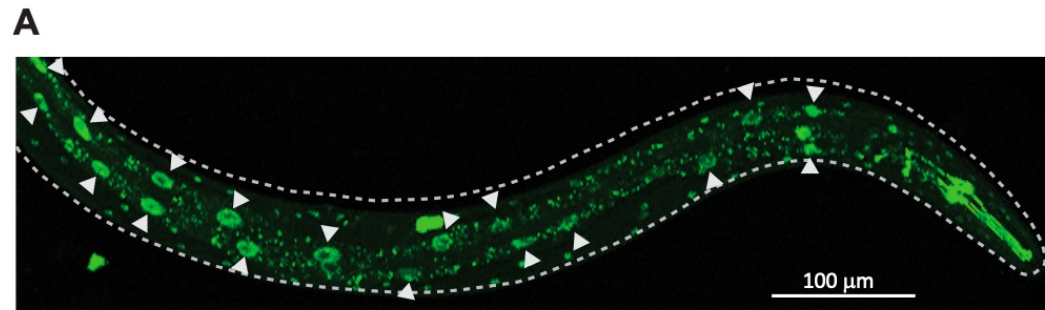
The HLH-11 Feedback Loop: Bridging Fat Oxidation with Mitochondrial Stress Responses and Lifespan

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Why HLH-11?

HLH-11 is the conserved transcription factor
→ the regulation of lipid metabolism, mitochondrial stress response, and lifespan in *C. elegans*

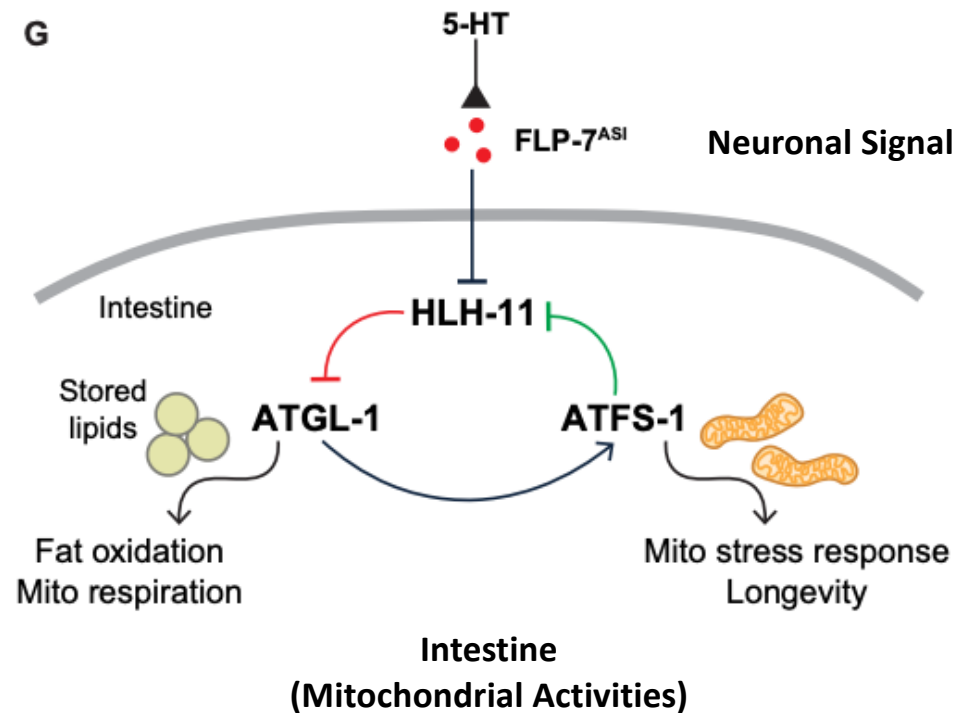


Phlh-11::hlh-11GFP

(Littlejohn et al., 2020)

Why HLH-11?

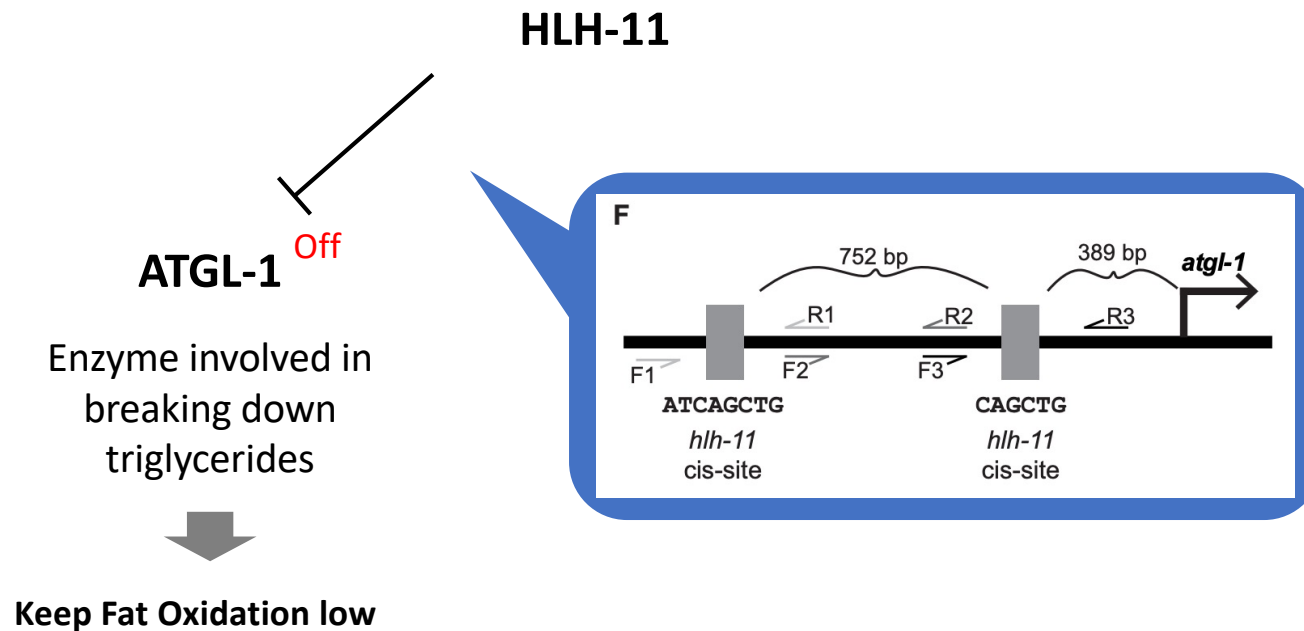
Feedback loop that coordinates fat oxidation with longevity.



(Littlejohn et al., 2020)

Why HLH-11?

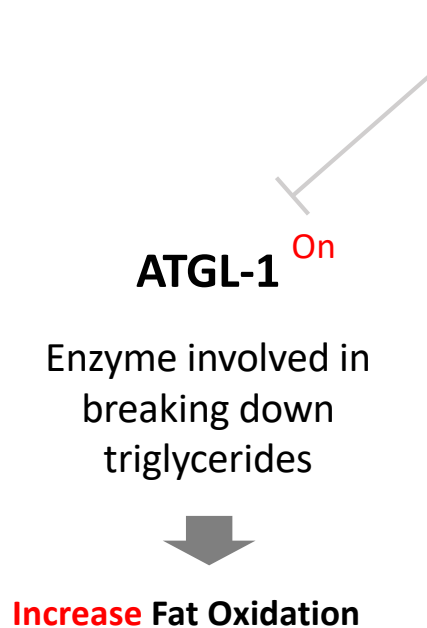
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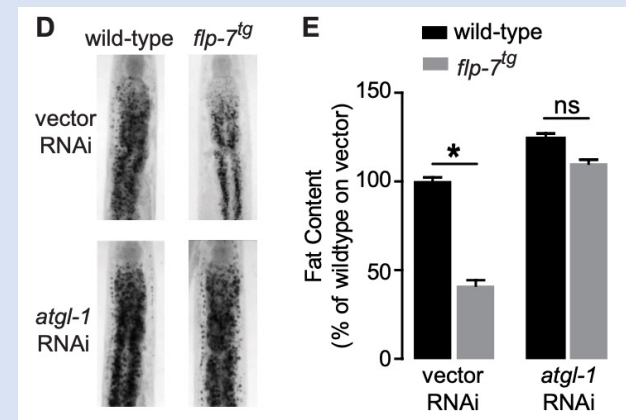
(Littlejohn et al., 2020)

Why HLH-11?

Feedback loop that coordinates fat oxidation with longevity.



Role of ATGL-1

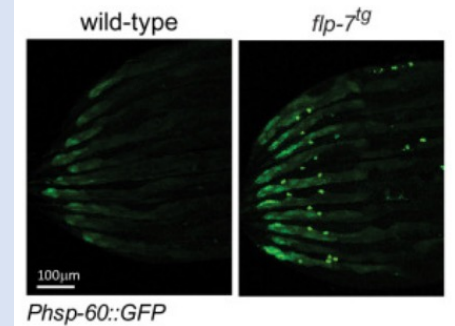
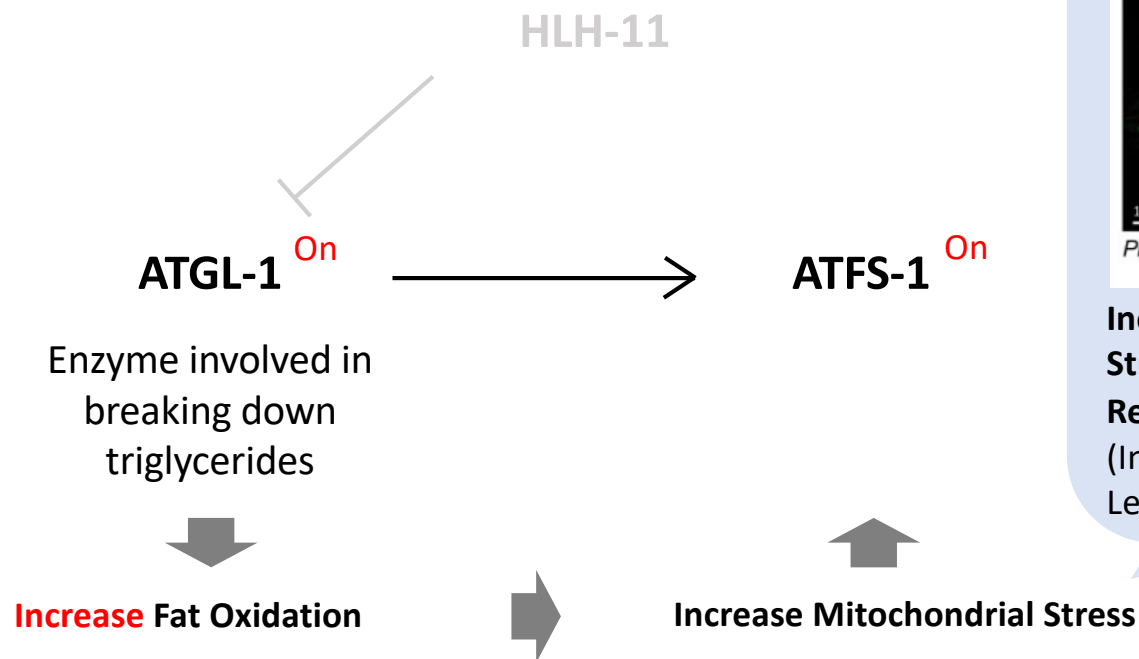


Enhanced fat oxidation observed in worms with an overactive FLP-7 system.

(Littlejohn et al., 2020)

Why HLH-11?

Feedback loop that coordinates fat oxidation with longevity.

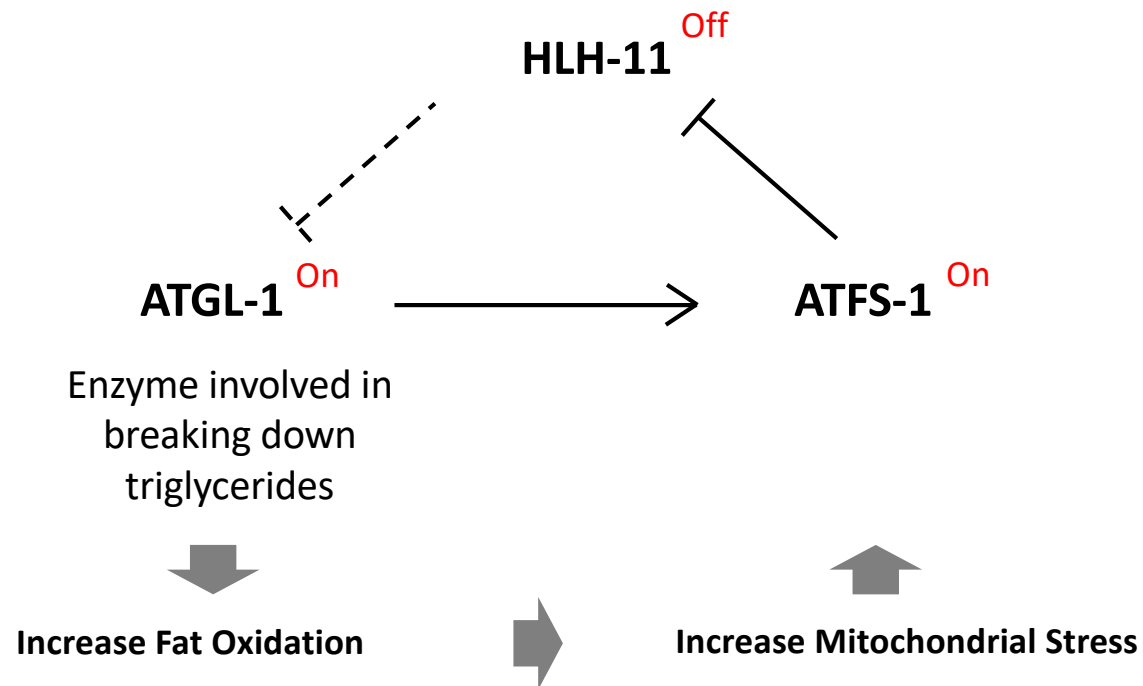


Increased Mitochondrial Stress upon HLH-11 Repression in *flp-7* Mutants
(Indicated by Elevated HSP-60 Levels)

(Littlejohn et al., 2020)

Why HLH-11?

Feedback loop that coordinates fat oxidation with longevity.

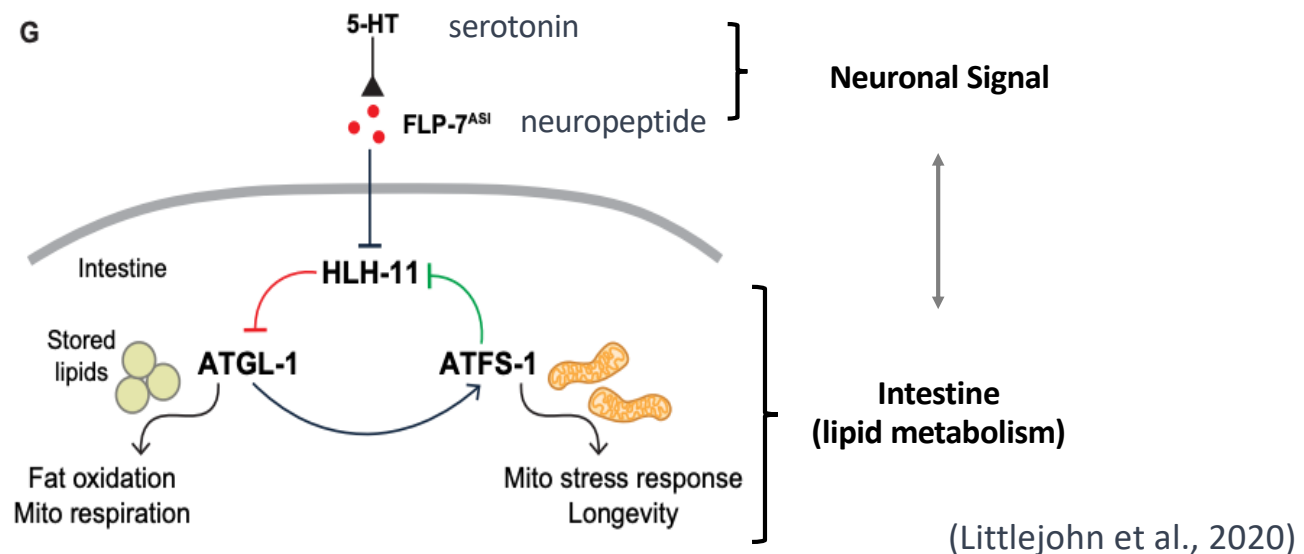


(Littlejohn et al., 2020)

Why HLH-11?

Feedback loop that coordinates fat oxidation with longevity.

→ “controlling hlh-11 levels can serve as an excellent surrogate for titrating intestinal fat stores in future efforts” (Littlejohn et al., 2020)



Research Focus & Project Outline

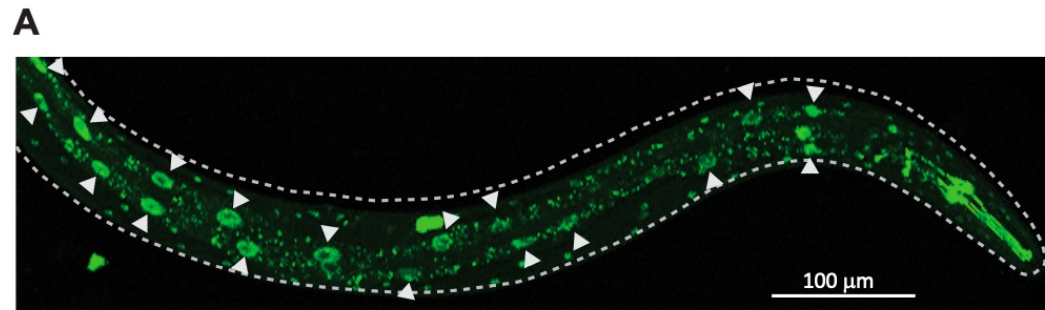
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Why Tissue-Specific hlh-11 Rescue Construct?

Dissecting Tissue-Specific hlh-11's Roles

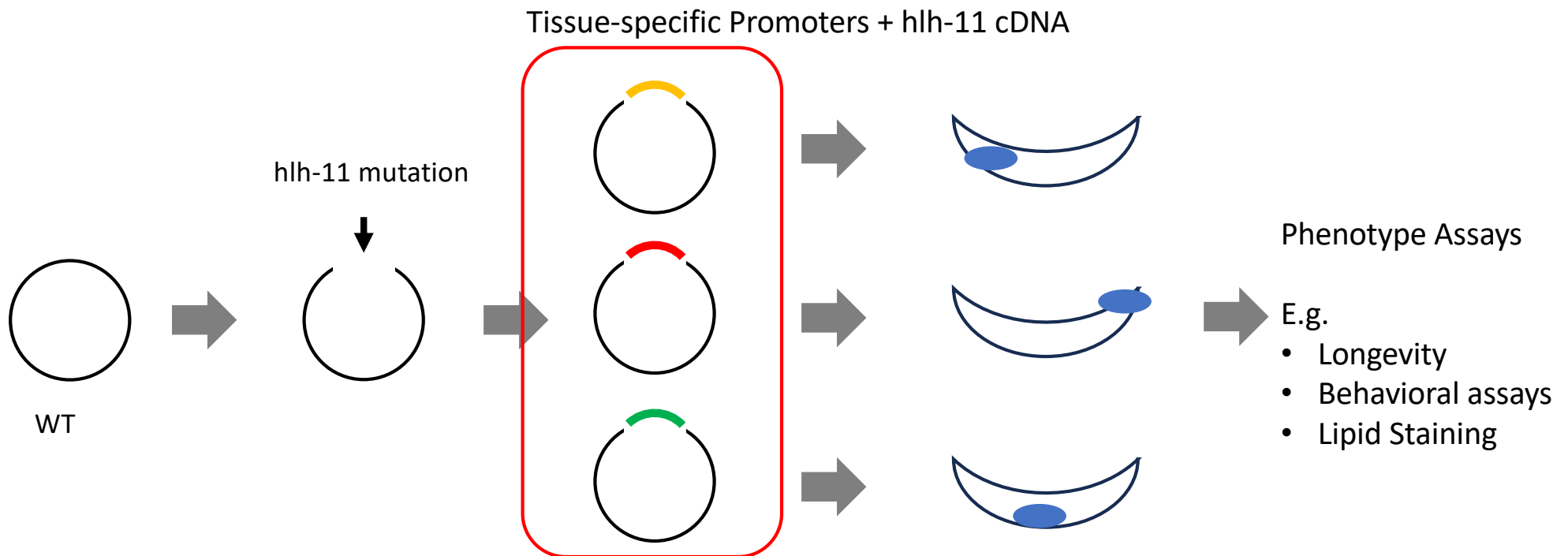


Phlh-11::hlh-11GFP

(Littlejohn et al., 2020)

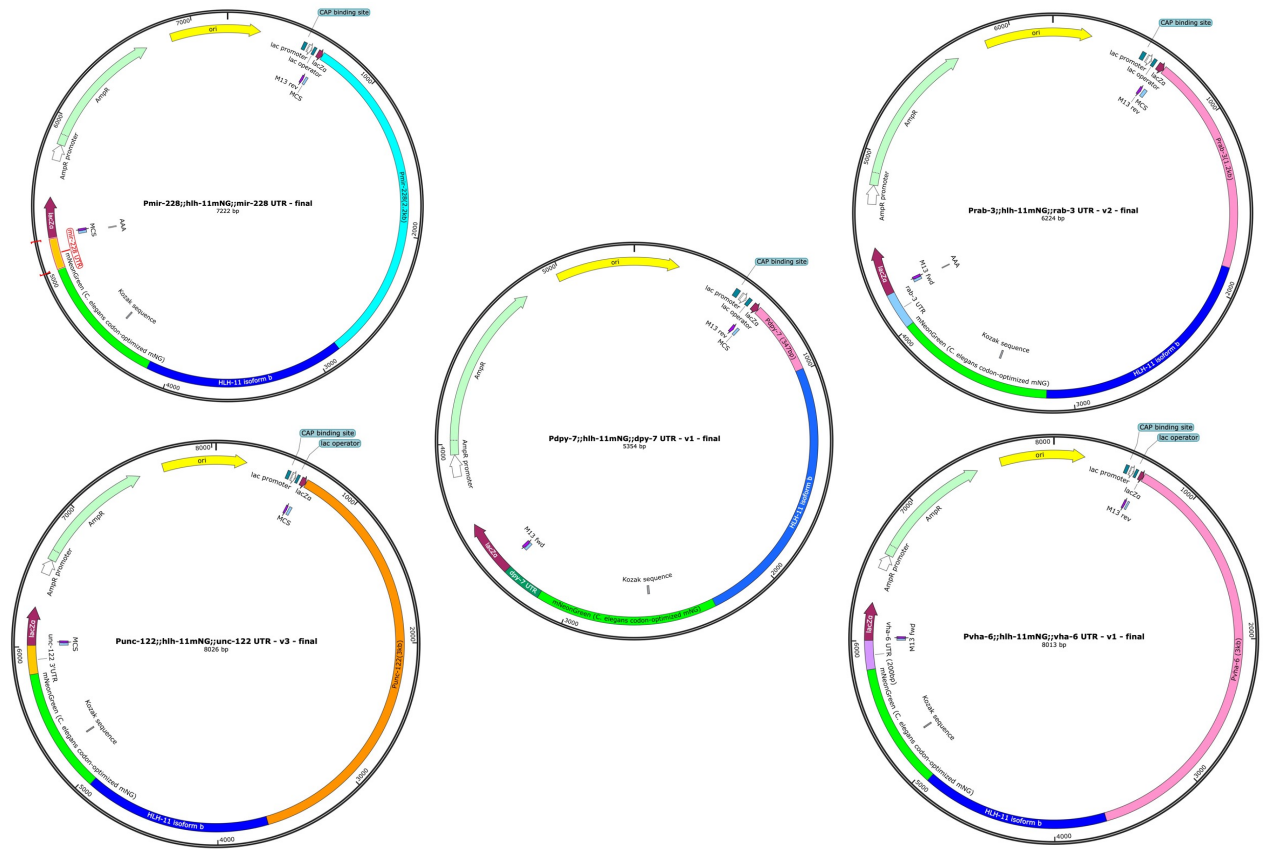
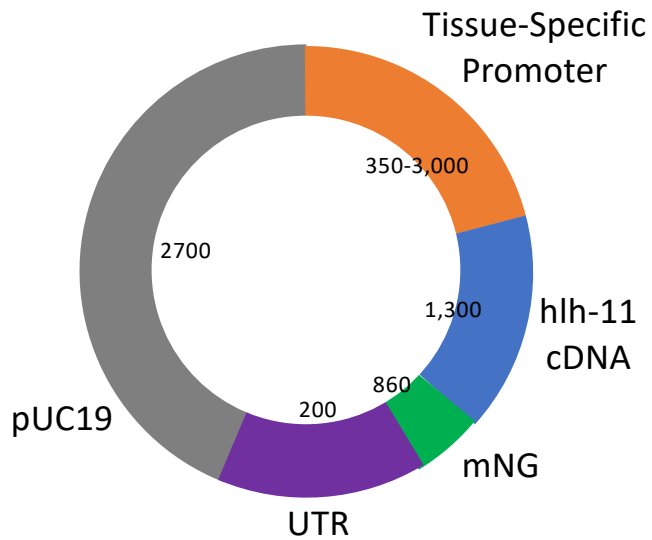
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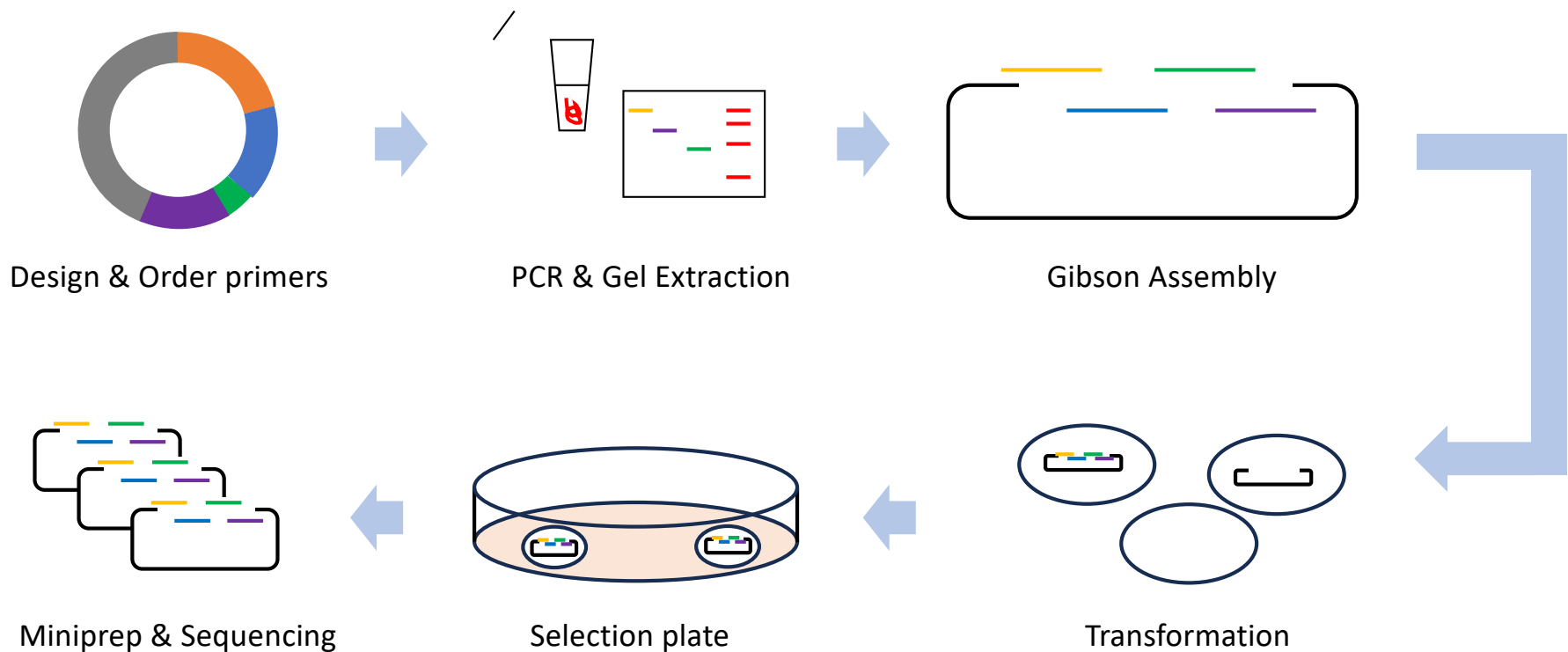
How Tissue-Specific hih-11 Rescue Construct?

Design:



How Tissue-Specific hlh-11 Rescue Construct?

Methodology:



How Tissue-Specific hlh-11 Rescue Construct?

Progress:

Promoter	Tissue	Status
Prab-3	Neuron	Completed (July 7)
Pmir-228	Glia	Completed (July 11)
Pdpy-7	Hypodermis (skin)	Completed (August 7)
Punc-122	Coelomocyte	Pending
Pvha-6	Intestine	Pending
	Phlh-11::hlh-11 mcherry	Not started

Research Focus & Project Outline

Research Focus: Exploring the functional connections between intestine and nervous system in *C. elegans*.

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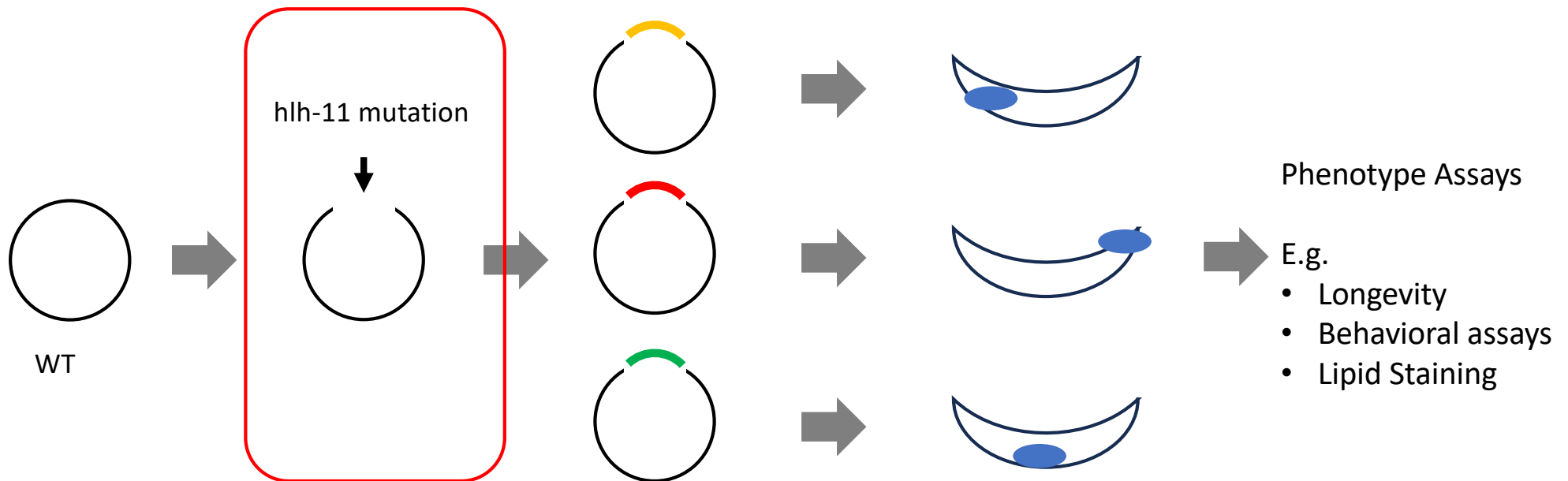
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Why hlh-11 CRISPR (Global Knockout)?

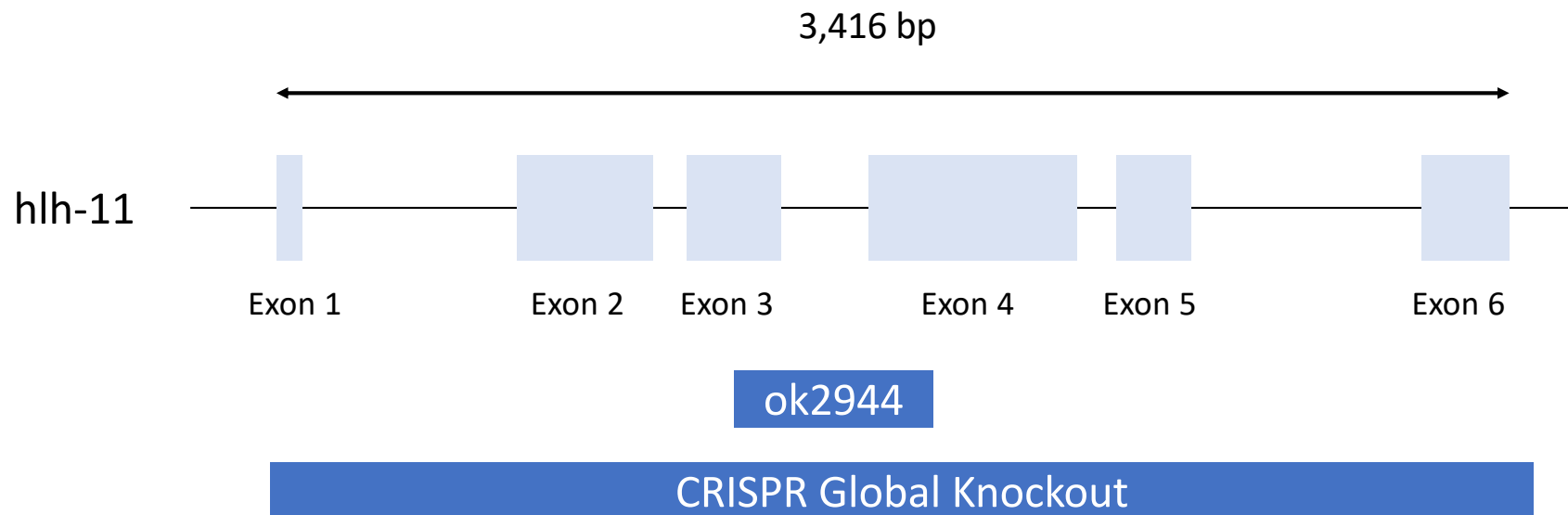
Dissecting Tissue-Specific hlh-11's Roles

Tissue-specific Promoters + hlh-11 cDNA



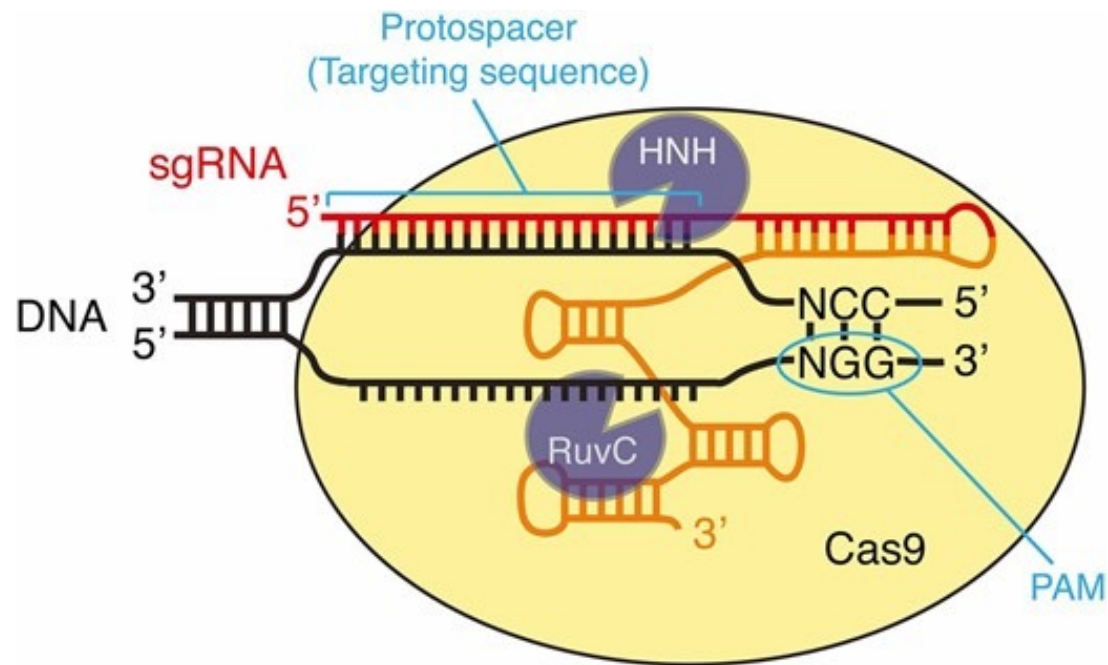
Why hlh-11 CRISPR (Global Knockout)?

Existing mutant *ok2944* deletes only parts of exons 3 & 4 of hlh-11
→ Global knockout targets all 6 exons for a complete knockout



How hlh-11 CRISPR (Global Knockout)?

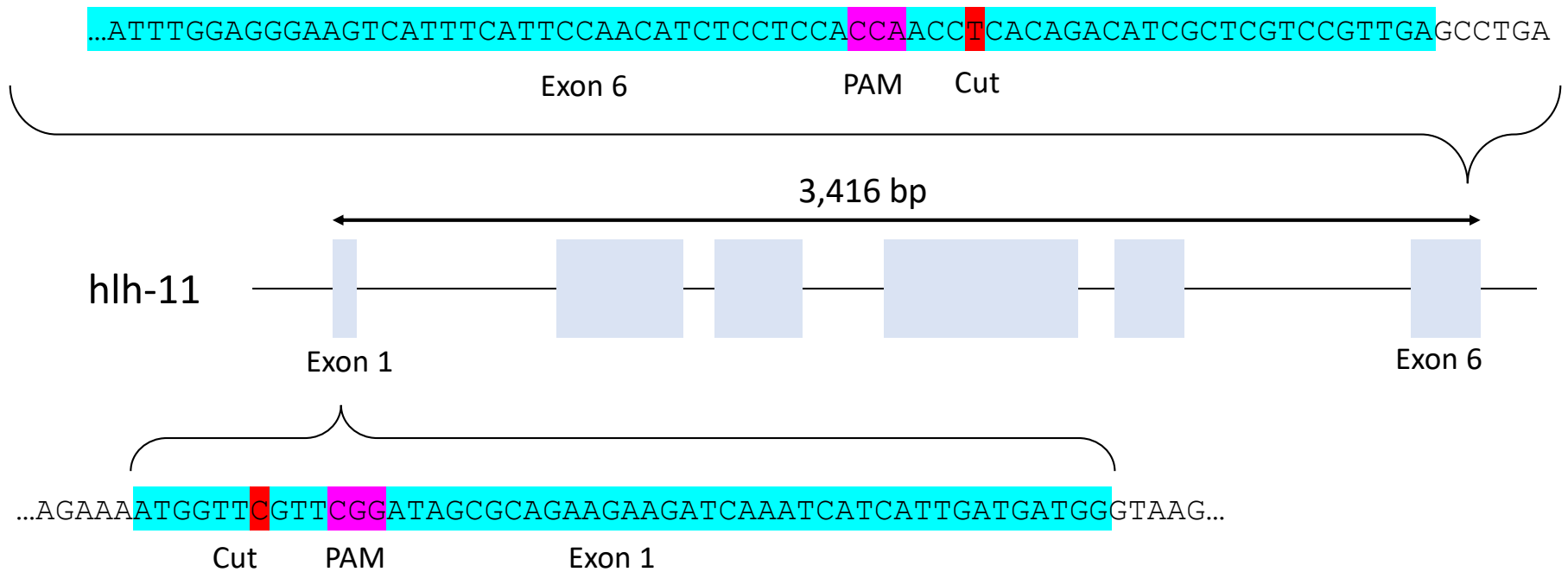
CRISPR Cas9



(Dickinson & Goldstein, 2016)

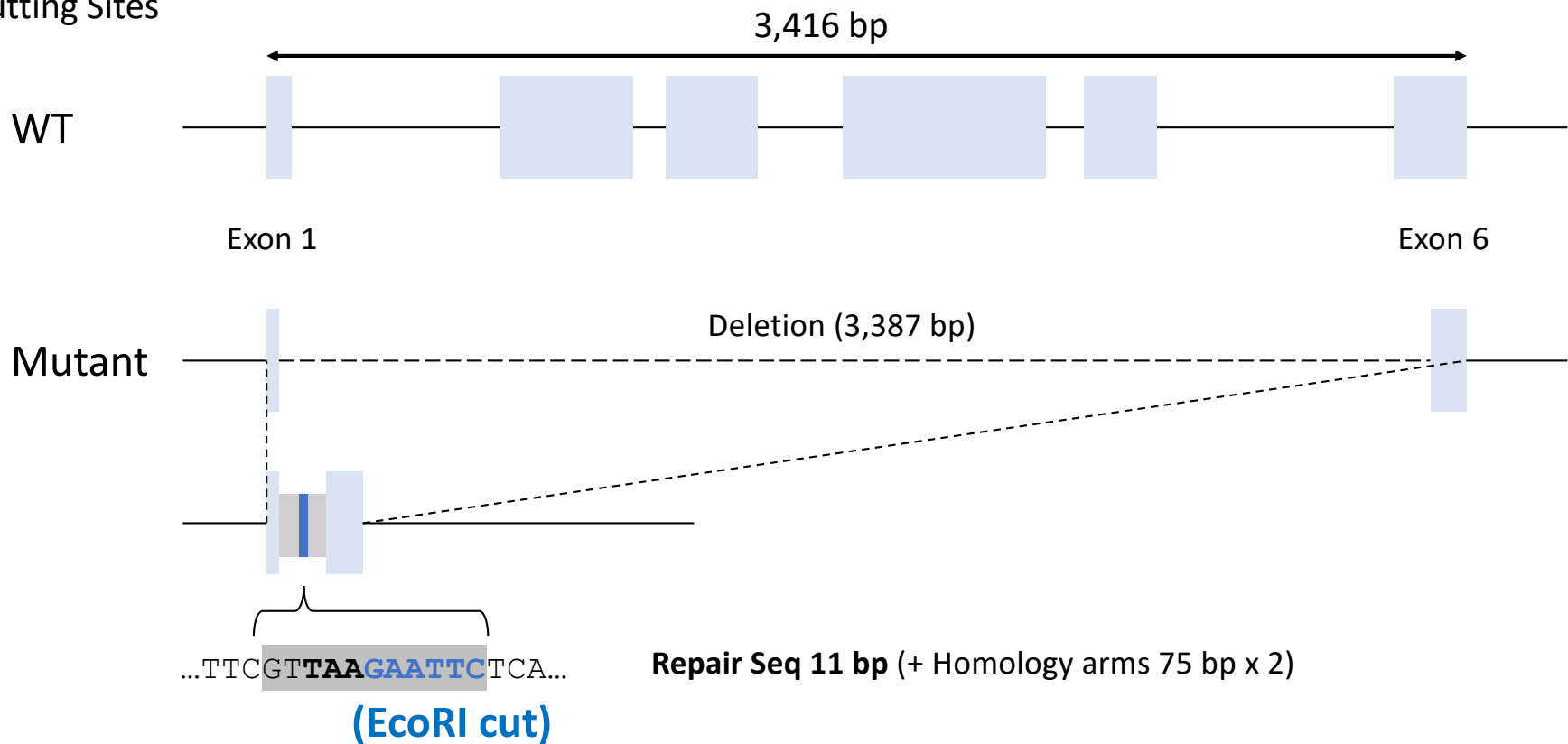
How hlh-11 CRISPR (Global Knockout)?

Design



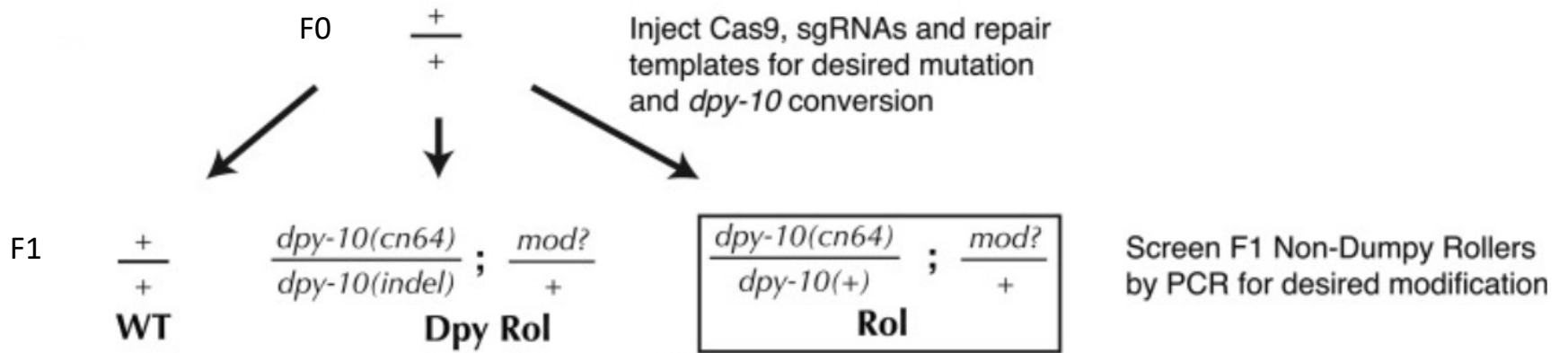
How hlh-11 CRISPR (Global Knockout)?

Cas9 Cutting Sites

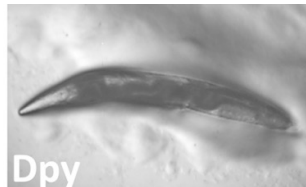
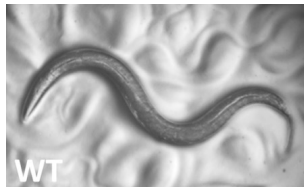


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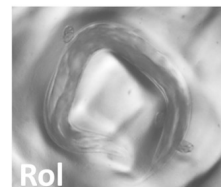
Screening Strategy (Co-CRISPR with marker mutation)



cn64 mutation has dominant Roller phenotype
 → eliminating the need for outcrossing to remove the marker mutation.



Shorter body



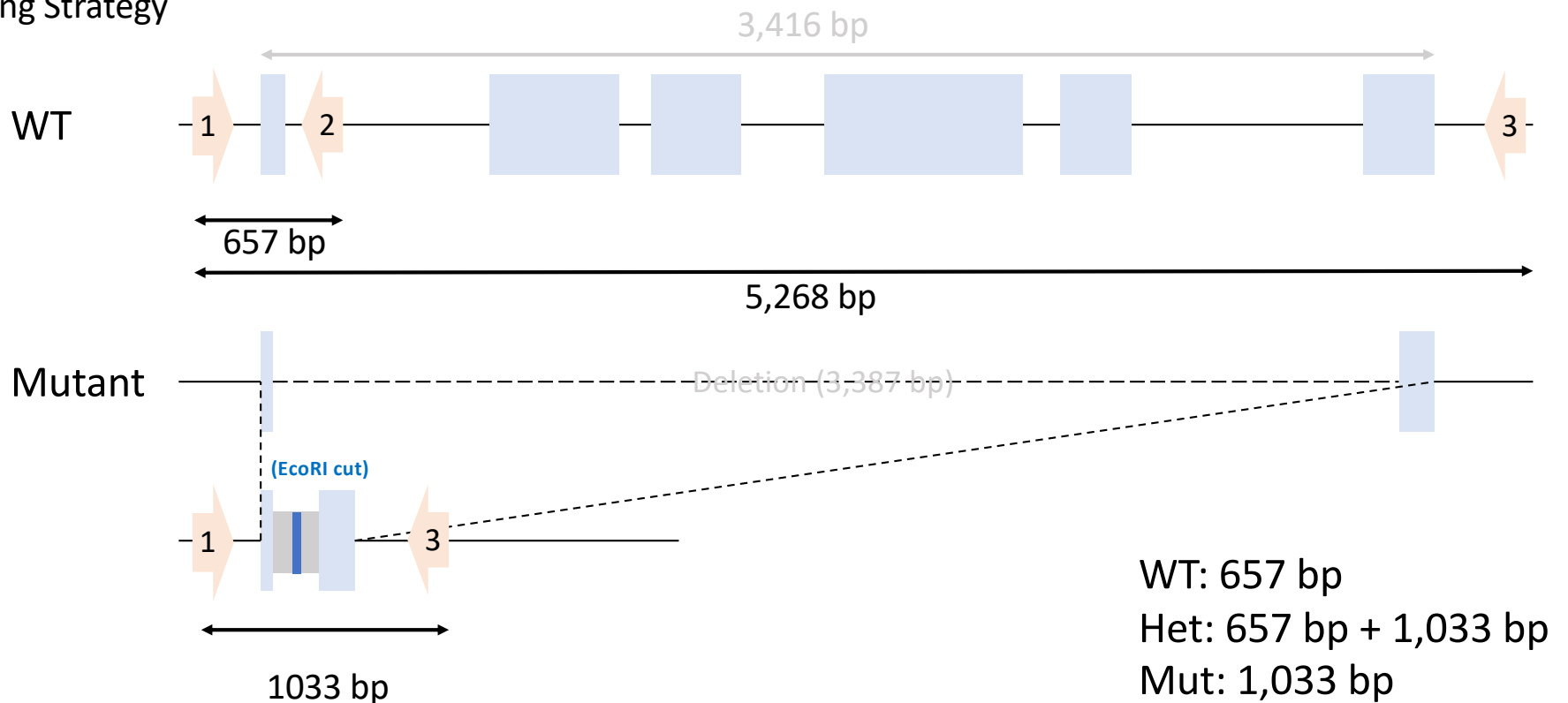
Rolling
(Spiral motion)

Genotyping

(Dickinson & Goldstein, 2016)

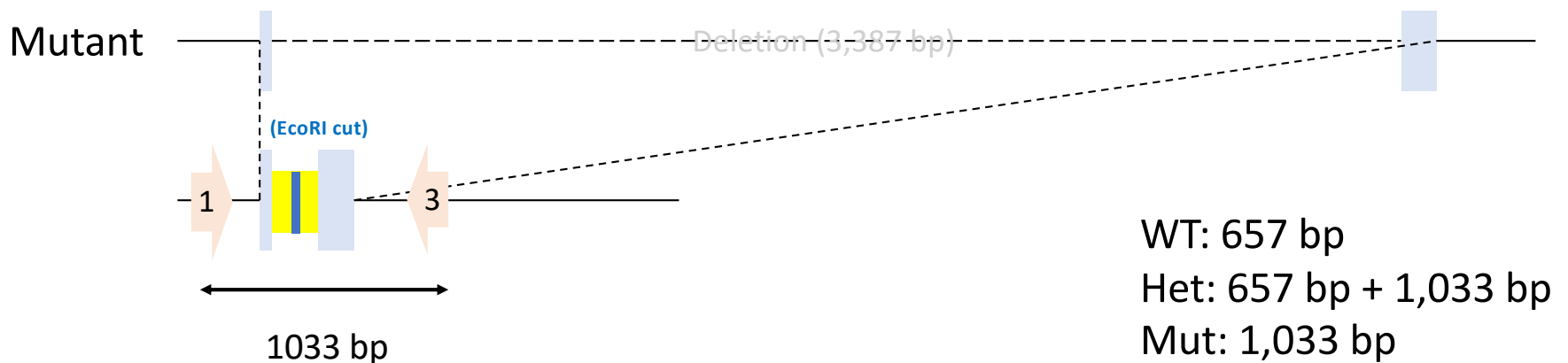
How hlh-11 CRISPR (Global Knockout)?

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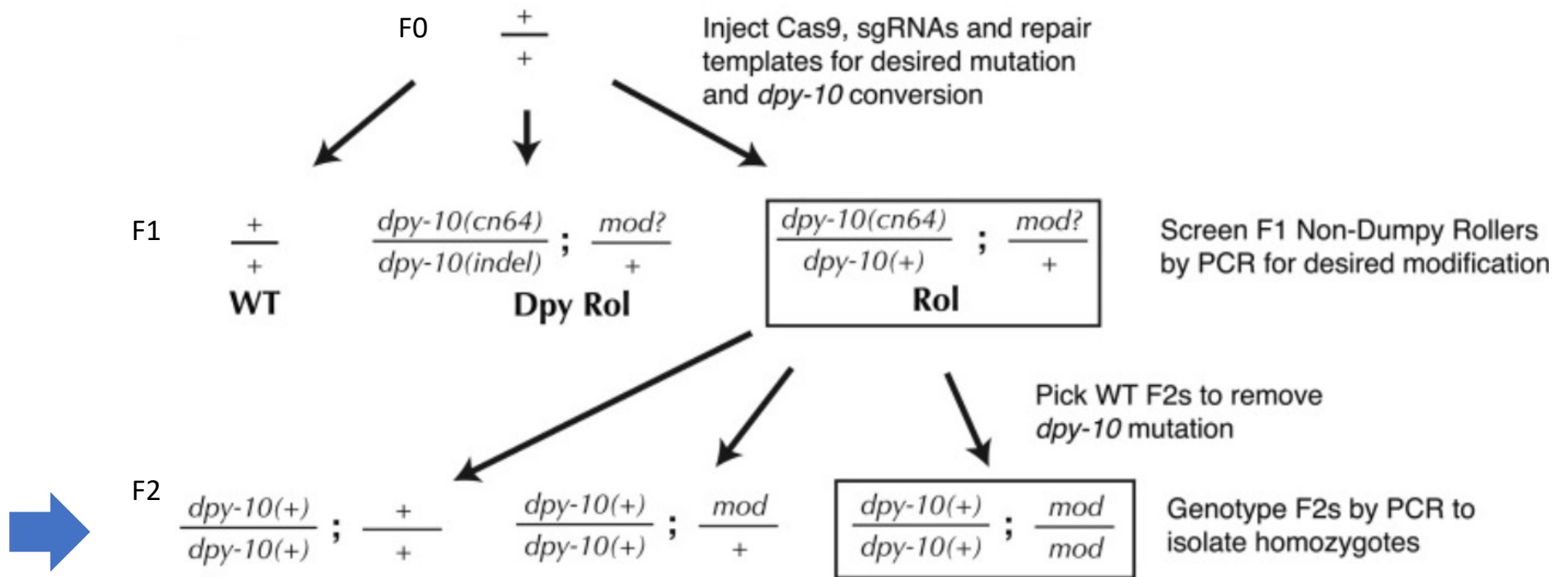
How hlh-11 CRISPR (Global Knockout)?

NNNNNNNCTNGNAAGAGCCTATCTTCTTTGATTCTACAAAGAAAATGGCATGTCAGCCAGTCAGCCAACATAGTGGGGGAAAGGTGATAAGAGTTACGGGTTTCGGGAA
ACTTTTGTTCGAAAAAGAGAGAATAAGGAACAAATAGAATTTCAATTCGAGAACCTCAAAAATCAAAAAAGAAAATTTTTTTCAGGTAGATCAATAGTAGAAAATGGTTCGT
TAAGAATTCACAGACATCGCTCGTCCGTTGAGCCTGACTTTTGACAAATGTAGAGTTACAATTACAAATACAATTATCAAATTTCCACGTTTCATAATCTCTCTCTCTTTCTCT
TTTTCTCTCTTTCTTTCTTCAGTTGATCCTGTTTTTGTCTTTCATCTTTTTCTCTCTCTCACCAGGCACAGTATCTATCATTTTTATCACAAAATTTGATGAATTTTTGTTCTTTT
CCCATTTTCTTCTTGTGTTTAGGTATACTACTTGTGTTGTTTCTATGAAATGTTTTTTCCAAAATTTTTGTGCCGATTGTGGTCTTCTTGTCAATTTTTCTTCTAATCAATCTAGCTT
CCTTATTTCCCGCGCTCTCACGGACCTATCCATTGTTTCGTTCTCCCACCGATAACTTTTTGTTTTTTCATTTTCCTTTTTTCCAGTCTTGTTCATCTCCAGACACTGCCCGTT
ACTTTTACACAAATCGTGTCGATTTCTCTTTTTTTCATTTTTTCAACCGGTGGTTTTGTTGACCGTATGTGCAGCTTGAAACACTTTCCNTTCCNACCTCENNTATGTCTCTCTAT
NGNTATCGCGCANNATCNACTAAATAATTTAANATAAACCCANAGNACNACCNCTGAGACAAGANTACCAATATTTATGTGAAAATTATGNNGATGATGANGANACAG
GACNNAN



How hh-11 CRISPR (Global Knockout)?

Screening Strategy



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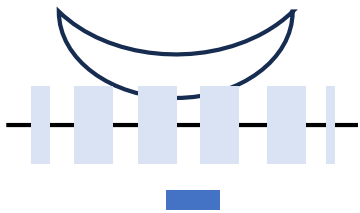
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hlh-11 mutant x Phlh-11::hlh-11 GFP



hlh-11 mutant (ok2944, male, #1681)



Phlh-11::hlh-11 GFP (hermaphrodites, #1698)



GFP



Genotyping

→ #

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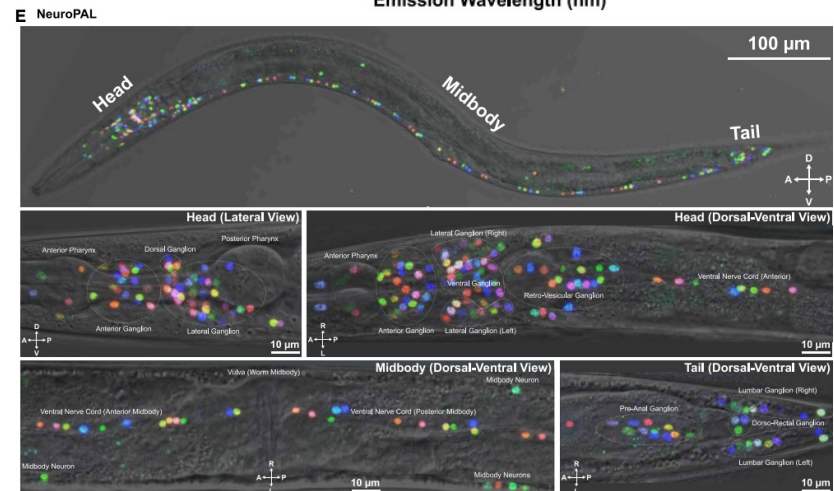
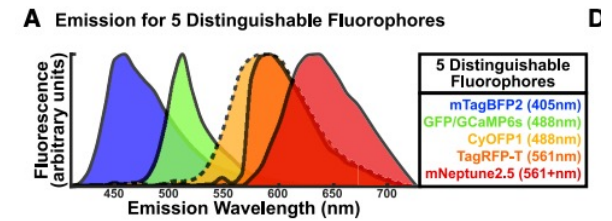
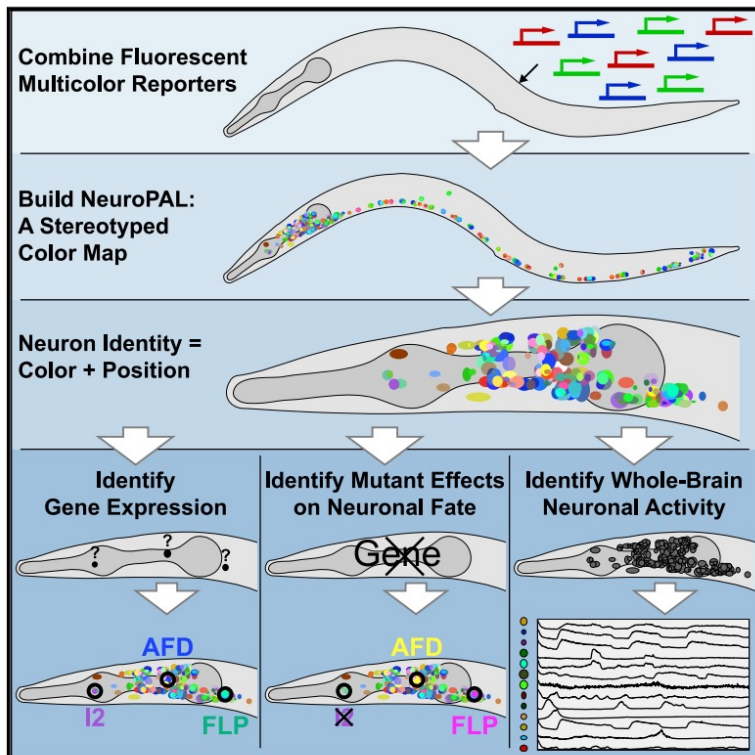
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What's NeuroPAL?

Graphical Abstract



(Yemini et al., 2021)

NeuroPAL x Phlh-11::hlh-11 GFP

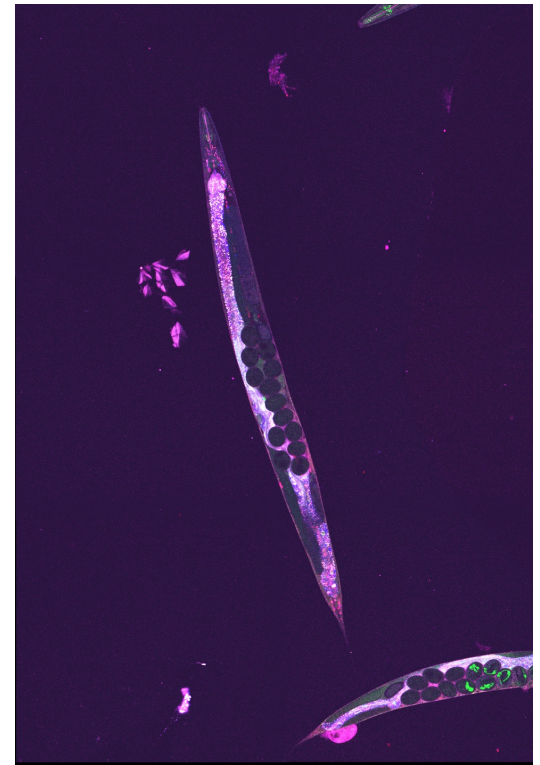


NeuroPAL



Phlh-11::hlh-11 GFP

A1 Confocal:
Kathy's Training (June 15 & 20)



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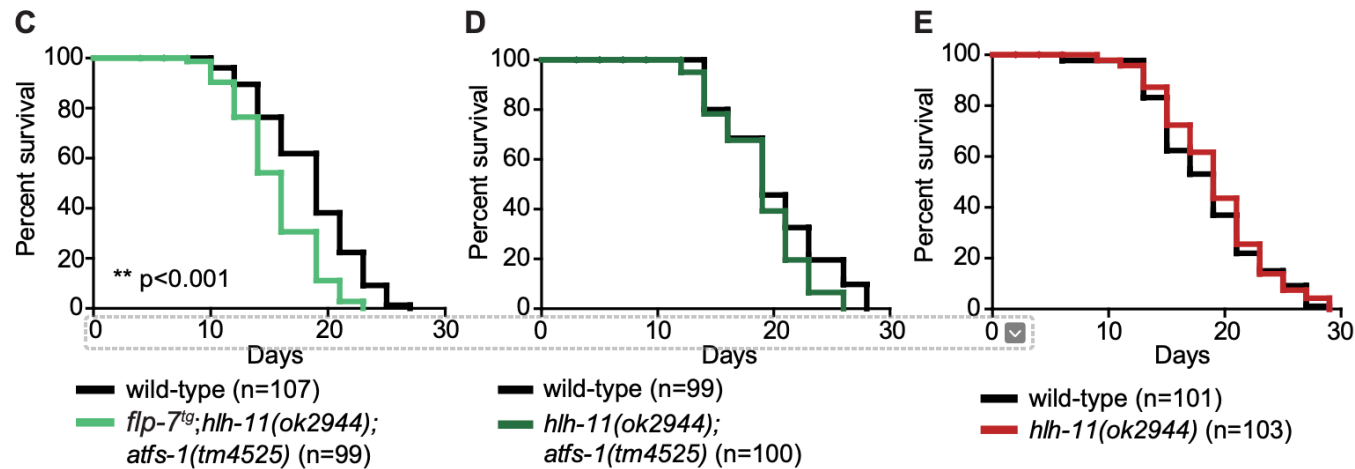
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Role of *hlh-11* in Lifespan

A slight increase in lifespan with *hlh-11* mutants (Littlejohn et al., 2020)



→ A larger sample size to provide more definitive insights (n=400)

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Future directions

1. Cloning tissue-specific hlh-11 rescue construct.
 - Cloning: CLM, Intestine / Injection / Phenotype Experiments
2. hlh-11 CRISPR (Global Knockout)
 - Replace ok2944 with CRISPR
3. Crossing of hlh-11 mutant with Phlh-11::hlh-11 GFP
4. NeuroPAL x hlh-11 GFP Imaging
 - We need more pics
5. Lifespan Experiment (with Esra).
 - Esra on-going



Srinivasan Lab

Supriya Srinivasan, Ph.D.
Chung-chi Liu, MS.
Anthony Perez, Ph.D.
Esra Karaca, Ph.D.
Ayushi Shah
Cassandra White
Matthew Lee
Grisha Tamazyan
Yijun Wang

Harvey Mudd College

Jae Hur, Ph.D.
Gabriela Gamiz
Danny Ledezma

Ben Huppe '14 Memorial
Internships Fellowship



Thank you!