Method to Culture + Assay Large-Scale Mixed-Stage *C. elegans* Populations for Omics Studies

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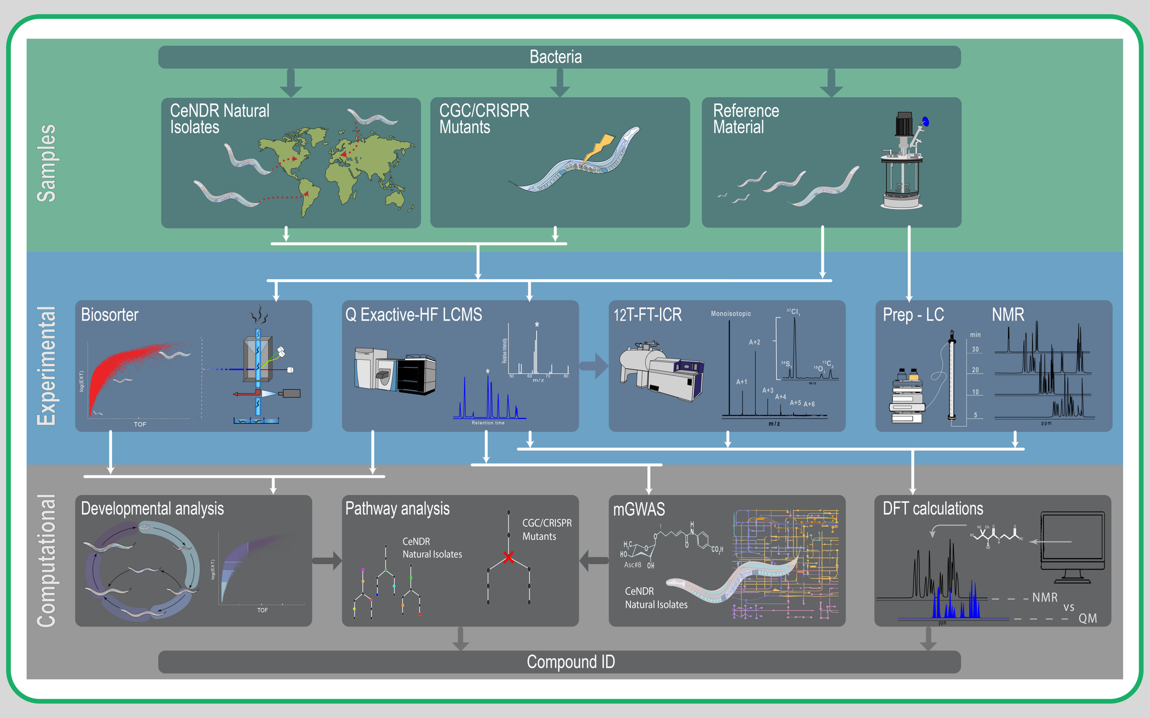
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Abstract

Caenorhabditis elegans (*C. elegans*) has been and remains a valuable model organism to study developmental biology, aging, neurobiology, and genetics. The large body of work on *C. elegans* makes it an ideal candidate to integrate into large-population, whole-animal studies to dissect the complex biological components and their relationships in a given organism. In order to use *C. elegans* in collaborative omics research, a method is needed to generate large populations of *C. elegans* where a single sample can be split and assayed across diverse platforms and instruments for comparative analyses.

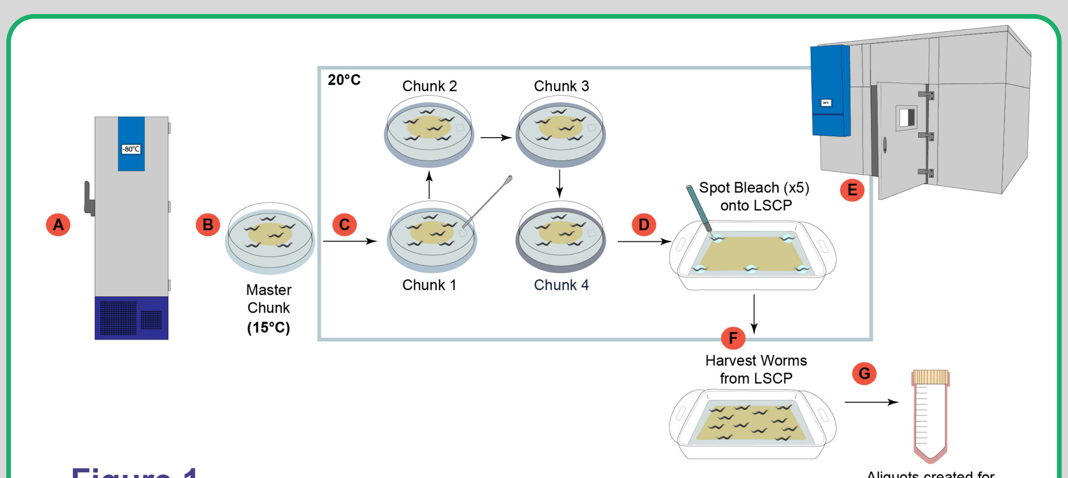
Here, **a method to culture and collect an abundant mixed-stage C. elegans population on a large-scale culture plate (LSCP) and subsequent phenotypic data** is presented. This pipeline yields sufficient numbers of animals to collect phenotypic and population data, along with any data needed for omics experiments (*i.e.* genomics, transcriptomics, proteomics, and metabolomics). In addition, the LSCP method requires minimal manipulation to the animals themselves, less user prep time, provides tight environmental control, and ensures that handling of each sample is consistent throughout the study for overall reproducibility. Lastly, methods to document population size, population distribution, and presence/absence of *C. elegans* life stages in a given LSCP are presented.

Overall Idea + Project Outline



Bacteria
Samples
CeNDR Natural Isolates
CGC/CRISPR Mutants
Reference Material
Experimental
Biosorter
Q Exactive-HF LCMS
12T-FT-ICR
Prep - LC
NMR
Computational
Developmental analysis
Pathway analysis
mGWAS
DFT calculations
Compound ID

Sample Growth Pipeline



20°C
Chunk 2
Chunk 3
Chunk 1
Chunk 4
Master Chunk (15°C)
Spot Bleach (x5) onto LSCP
Harvest Worms from LSCP
Aliquots created for desired applications

Figure 1

Figure 1 | Overview of the Large Scale Culture Plate (LSCP) sample growth pipeline:

(A) Once received in the lab, all strains are prepared and frozen for long-term storage at -80°C

(B) A "master chunk" plate is prepared from a frozen worm stock and stored at 15°C to be used for no longer than one month.

(C) Each sample goes through four successive chunking steps to reduce generational stress prior to growing on the LSCP.

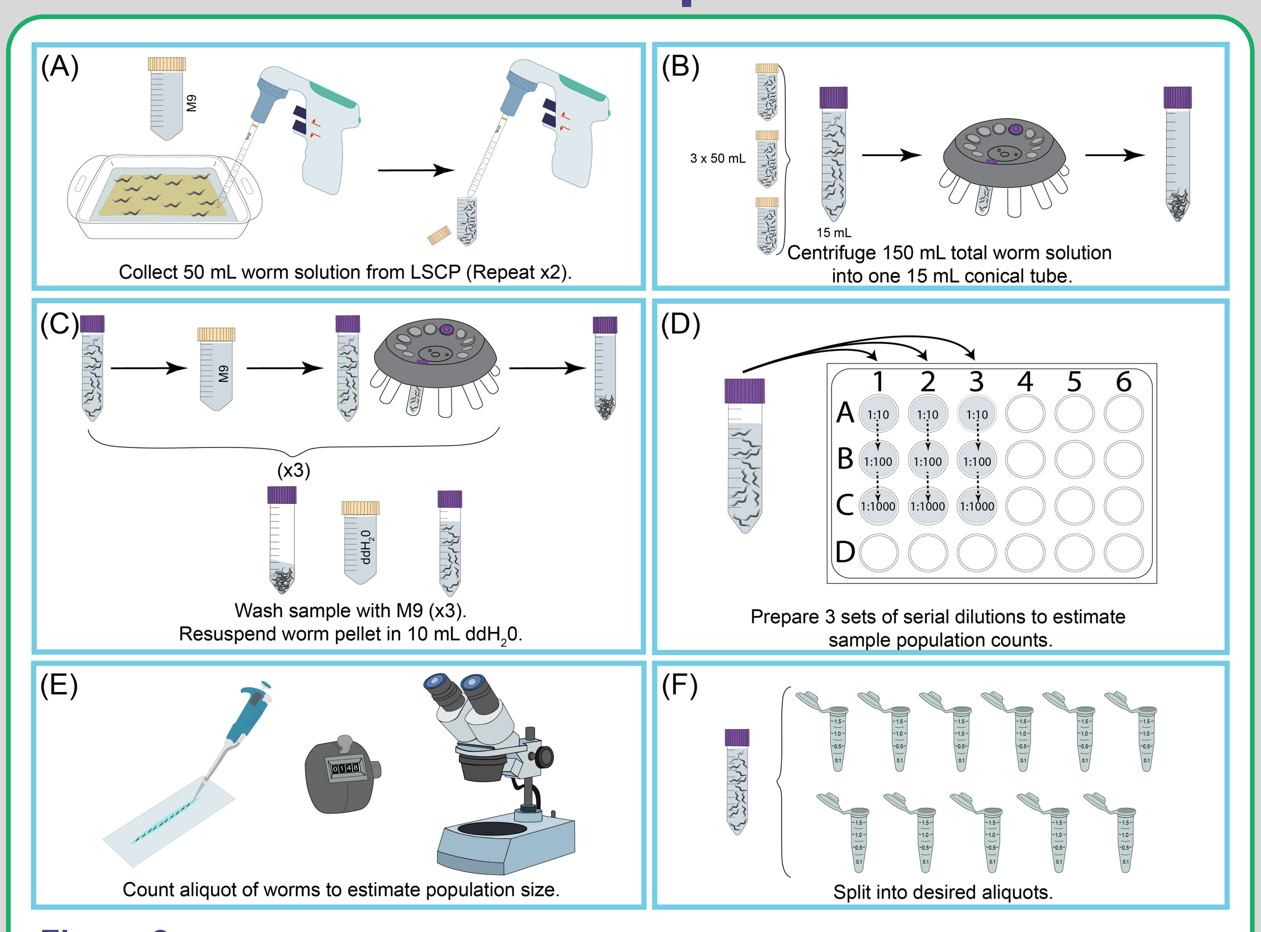
(D) Five individual gravid adults are picked from the "chunk 4" 6 cm plate in Step (D) and spot bleached on five given areas of the LSCP.

(E) The LSCP is placed in a Controlled Temperature room to grow at 20°C until it has become full of adult worms, reached a large population size, and has minimal bacteria left.

(F) The worm population is harvested and collected for downstream steps.

(G) Aliquots are created from LSCP and are flash frozen for downstream desired applications.

Harvest Collection Pipeline



(A)
Collect 50 mL worm solution from LSCP (Repeat x2).
(B)
3 x 50 mL
15 mL
Centrifuge 150 mL total worm solution into one 15 mL conical tube.
(C)
(x3)
Wash sample with M9 (x3).
Resuspend worm pellet in 10 mL ddH$_2$0.
(D)
Prepare 3 sets of serial dilutions to estimate sample population counts.
(E)
Count aliquot of worms to estimate population size.
(F)
Split into desired aliquots.

Figure 2

Figure 2 | Overview of LSCP harvesting and estimating population size:

(A) Use 50 mL of M9 to wash worms off the NGMA surface and pipette worm suspension into a 50 mL conical tube. Repeat twice.

(B) Pour 15 mL of worm suspension into a new 15 mL conical tube. Pellet worms by centrifuging. Aspirate off M9 + debris without disturbing worm pellet. Repeat until all 150 mL of worm suspension are collected.

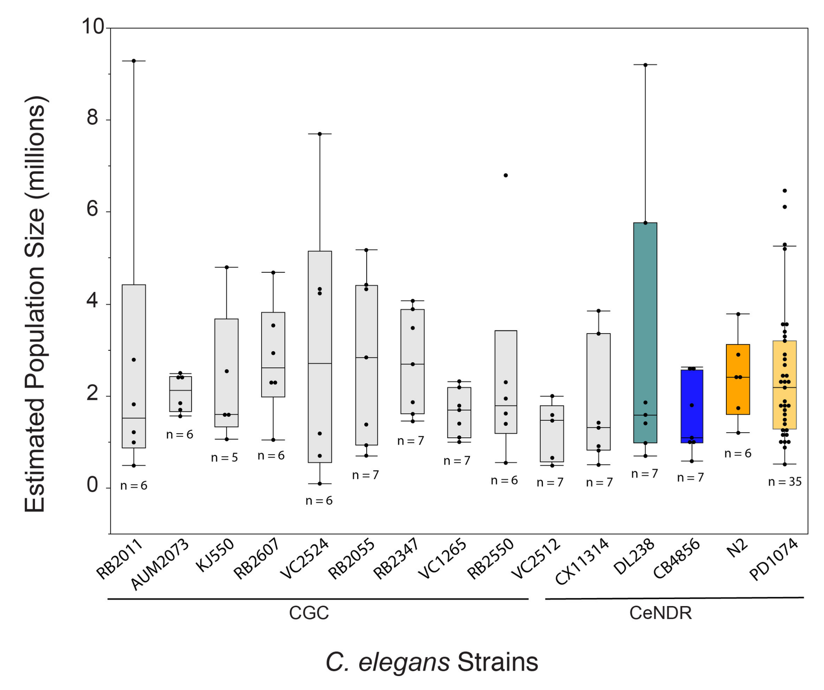
(C) Wash and centrifuge the worm pellet three times with M9 to get rid of any remaining debris. Once the sample is clean, resuspend worm pellet in 10 mL of ddH20.

(D) Create a serial dilution of sample to estimate worm population size. Choose the dilution that allows you to count worms most accurately. The dilution used may change depending on the population size of the LSCP. Once a dilution is chosen, ensure you count worms from all three aliquot replicates of that dilution.

(E) Aliquot the sample onto a clean slide and count worms present under a dissecting microscope.

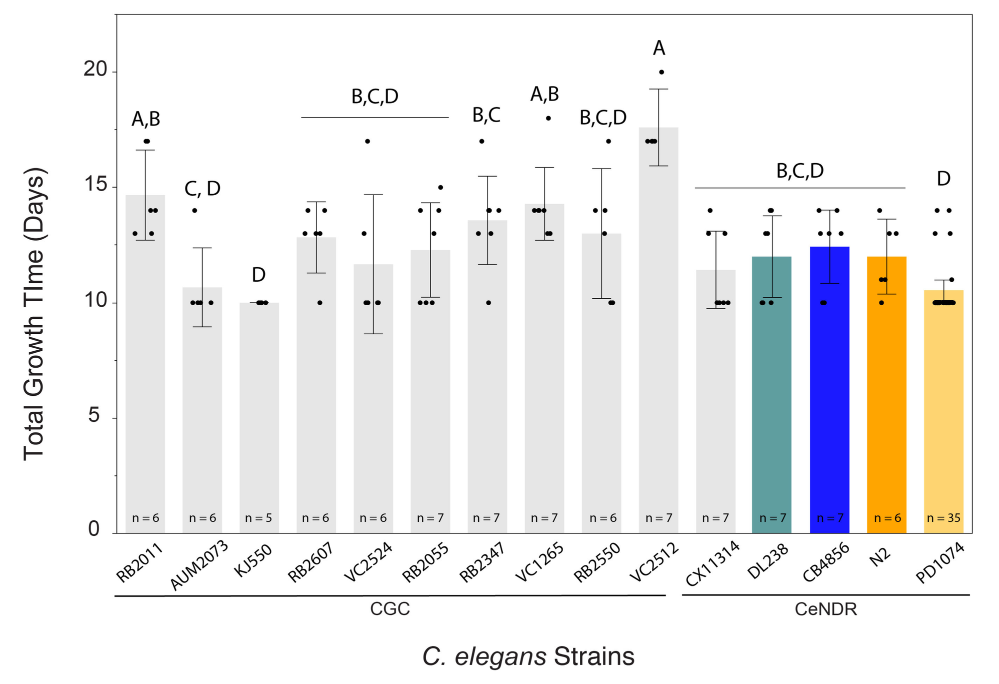
(F) Split sample into appropriate-sized aliquots.

LSCP Method Generates ~ 2.4 Million Mixed-Stage Worms in 10 - 20 Days



Estimated Population Size (millions)
C. elegans Strains
CGC
CeNDR
RB2011
AUM2073
KJ550
RB2607
VC2524
RB2055
RB2347
VC1265
RB2550
VC2512
CX11314
DL238
CB4856
N2
PD1074

Figure 3



Total Growth Time (Days)
C. elegans Strains
CGC
CeNDR

Figure 4

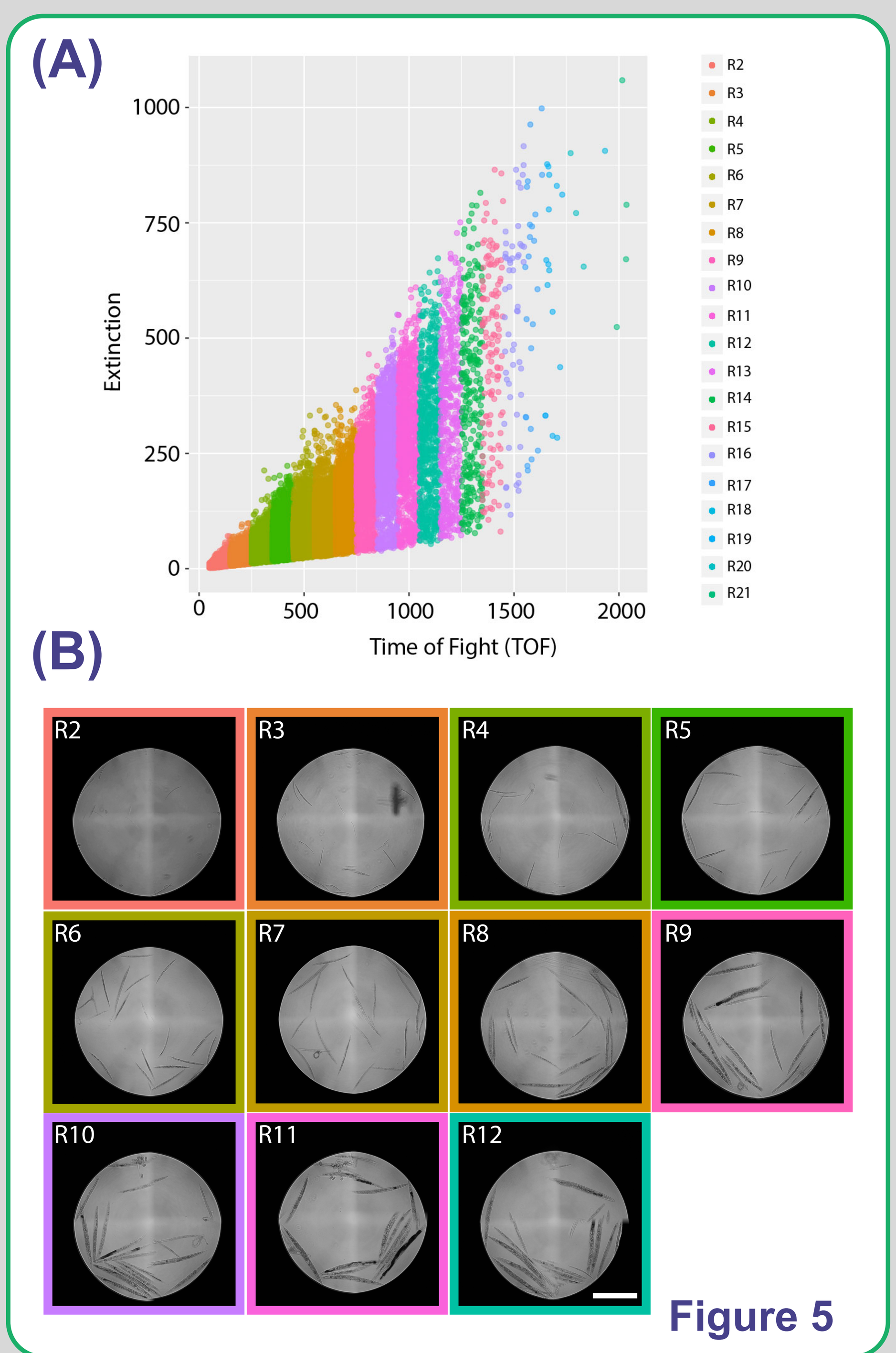
Figure 3 | LSCP method generates on average a population of 2.4 million mixed-stage worms

The LSCP yields population sizes in the smallest population growths at around 94,500 and at the biggest population growths at around 9,290,000. The mean population size across all strains was 2.4 million worms. Bars underneath C. elegans strain names indicate whether a strain is a CGC mutant or CeNDR natural isolate. LSCP sample size is displayed for each strain. Comparisons for all pairs using Tukey's HSD Test were performed. No significant differences were observed between estimated population sizes across *C. elegans* strains ($F(14,108) = 0.7$, $p = 0.77$). Colored bars indicate standard color displays for respective *C. elegans* strain representation. .

Figure 4 | LSCP method generates large mixed-stage populations of worms in 10 – 20 days

C. elegans LSCP grew until the sample was full of adult worms, reached a large population size, and had minimal bacterial left. LSCPs took between 10 – 20 days to grow to a full mixed-stage population, depending on the strain. The mean growth time across the strains was 12.2 days. LSCP sample size is displayed for each strain. Each error bar was constructed using 1 standard deviation from the mean. Levels not connected by same letter are significantly different. Comparisons for all pairs using Tukey's HSD Test. A significant difference was found in the amount of growth time on LSCP needed across *C. elegans* strains ($F(14,108) = 8.8$, $p < 0.0001$*). Colored bars indicate standard color displays for respective *C. elegans* strain representation.

Mixed Population + Growth Measurements of PD1074



(A)
Extinction
Time of Fight (TOF)
(B)
R2
R3
R4
R5
R6
R7
R8
R9
R10
R11
R12

Figure 5

Figure 5 | Mixed population and growth measurement of the wild-type reference strain, PD1074

(A) A representative LPFC distribution of one LSCP growth of the wild-type reference strain, a variant of the original N2 Bristol strain, (PD1074) documents the size distribution and event counts of a mixed-stage population. The x-axis displays the length (Time of Flight, TOF) of the object sorted. The y-axis displays the optical density (optical extinction, EXT) of the object sorted. Each data point is an object that was documented in the worm sample. Each TOF region that was used for image analysis is displayed in a different color. Twenty TOF regions were created (R2 – R21) ranging from a TOF of 50 to 2050.

(B) Images of worms sorted from TOF regions ranging from R2 – R12 are shown for this LPFC distribution. In region R2, L1 worms can be identified and in region R9 predominately gravid adults are identified spanning the two developmental larval extremes giving us approximate regions within the flow cytometer distribution of where stagesare expected in the distribution. Scale bar represents 1 mm.

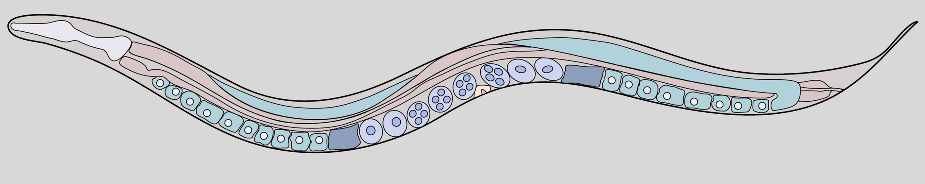
Takeaways

- Growth of *C. elegans* using the LSCP method yields an average of approximately 2.4 million mixed-stage worms per sample over the course of an average of 12.2 LSCP growth days.
- Growth of *C. elegans* using the LSCP method enables users to generate large mixed-stage populations of *C. elegans* with little handling and manipulation of the animals, which is ideal for large-scale omics studies
- With this LSCP method, users can easily integrate new strains of interest into a study with little knowledge of developmental timing and background expertise.
- Large particle flow cytometry and imaging of samples shows this method produces large mixed-stage populations of *C. elegans*

Ongoing Work

- *C. elegans* strains with mutations in the TOR pathway are currently going through our growth pipeline.
- NMR Metabolomics data has been collected and is currently being analyzed to identify consistencies in metabolism both within and across *C. elegans* strains and to find novel metabolites and features.
- This work is featured in a manuscript currently under review with JoVE.

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Acknowledgements

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