Video Article

# Plate-based Large-scale Cultivation of *Caenorhabditis elegans*: Sample Preparation for the Study of Metabolic Alterations in Diabetes

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## Abstract

Culturing *Caenorhabditis elegans* (*C. elegans*) in a large-scale manner on agar plates can be time-consuming and difficult. This protocol describes a simple and inexpensive method to obtain a large number of animals for the isolation of proteins to proceed with a western blot, mass spectrometry, or further proteomics analyses. Furthermore, an increase of nematode numbers for immunostainings and the integration of multiple analyses under the same culturing conditions can easily be achieved. Additionally, a transfer between plates with different experimental conditions is facilitated. Common techniques in plate culture involve the transfer of a single *C. elegans* using a platinum wire and the transfer of populated agar chunks using a scalpel. However, with increasing nematode numbers, these techniques become overly time-consuming. This protocol describes the large-scale culture of *C. elegans* including numerous steps to minimize the impact of the sample preparation on the physiology of the worm. Fluid and shear stress can alter the lifespan of and metabolic processes in *C. elegans*, thus requiring a detailed description of the critical steps in order to retrieve reliable and reproducible results. *C. elegans* is a model organism, consisting of neuronal cells for up to one-third, but lacking blood vessels, thus providing the possibility to investigate solely neuronal alterations independent of vascular control. Recently, early neurodegeneration in diabetic retinopathy was found prior to vascular alterations. Thus, *C. elegans* is of special interest for studying general mechanisms of diabetic complications. For example, an increased formation of advanced glycation end products (AGEs) and reactive oxygen species (ROS) is observed, which are reproducibly found in *C. elegans*. Protocols to handle samples of adequate size for a broader spectrum of investigations are presented here, exemplified by the study of diabetes-induced biochemical alterations. In general, this protocol can be useful for studies requiring large *C. elegans* numbers and in which liquid culture is not suitable.

## Video Link

The video component of this article can be found at https://www.jove.com/video/58117/

## Introduction

Protein analyses, such as a western blot or mass spectrometry, require milligrams of protein. This yield requires a large-scale culturing of hundreds of *C. elegans*, which can be accomplished either by liquid culture or on solid media transferring the nematodes by washing. Fluid and shear stress induces the expression of epithelial sodium channels (ENaC), which could increase the osmotic stress through an increased uptake of sodium, potentially altering the lifespan of *C. elegans* and affecting metabolic analyses[1]. Therefore, some critical steps in this protocol for the plate-based approach take the reduction of stress affecting experimental variability into account. Liquid culture, on the other hand, influences the phenotype of the nematodes and complicates the culture and collection of an exact number of nematodes[2]. Moreover, reactive substances can be altered by media components and may distribute unevenly before reaching the nematodes. Regarding the limitations of liquid culture, this protocol provides an alternative approach to culturing large-scale samples of *C. elegans*.

*C. elegans* is a model organism with a distinct network of 302 neuronal cells, making up one-third of all its cells[3]. Since its introduction into science, many homologous and orthologous genes have been described, amplifying its value as a model for medical research. Recently, evidence for neurological impairment in diabetic retinopathy, preceding vascular damage, has been presented[4]. *C. elegans* is lacking blood vessels, but contains a distinct neuronal network, making it a suitable model to investigate neuronal alterations apart from vascular ones. Thus, *C. elegans* is of special interest for studying general mechanisms of diabetic complications. Biochemical alterations in diabetic complications involve the formation of AGEs, which further influence the formation of ROS in response to hyperglycemia[5]. AGEs are found in *C. elegans* and contribute to neuronal damage[6]. Chronic diseases are often caused by complex, polygenic processes requiring a multiparametric approach for the assessment of their underlying mechanisms, as exemplified here with the assessment of diabetic complications. This protocol can be of

 

use for obtaining multiple parameters simultaneously, as well as subsequently. Increased comparability and reproducibility of a multiparametric approach can be achieved by omitting the morphological and metabolic differences between liquid and solid media culture.

## Protocol

NOTE: This protocol is divided into five sections. In sections 1–3, the main protocol to culture *C. elegans* at a large-scale is presented. Sections 4 and 5 provide additional protocols for the assessment of exemplified metabolites occurring in diabetic metabolites. In detail, section 1 describes a general large-scale culture on plates. Section 2 focuses on the transfer of large amounts of *C. elegans*, whereas section 3 explains the harvesting of a large-scale sample. Section 4 explains the protein isolation from the sample and section 5 describes the sample preparation for subsequent liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses.

## 1. Large-scale Culture on Plates

1. In general, work under a sterile hood or next to an open flame to avoid contaminations.
2. **To culture worms until they reach the young adult stage, follow this previously published protocol[7] with the following adaptations.**
    1. Use living *Escherichia coli* OP50 bacteria to feed the nematodes and prepare 400 µM fluorodeoxyuridine agar (not ampicillin/FUdR) for experiments.
    2. For the preparation of a synchronous population, use unconcentrated OP50, and for succeeding experiments with the adults, use 3.3x concentrated OP50 by removing 70% of the supernatant.
    3. Daily feeding intervals are recommended. When assessing oxidative stress, different intervals and volumes for feeding might interfere with the results.
    4. For inoculation, take a plate with several nematodes and cut it into pieces of approximately 0.5 x 1 $cm^2$ with a scalpel. Transfer two to three pieces onto nematode growth media (NGM) plates of 60 mm in diameter, containing unconcentrated OP50.
    5. Incubate the plates at the temperature suitable for the strain (wild-type N2 should be cultured at 20 °C) until eggs are visible on the plate.
    6. Wash off most of the eggs and adult animals with 2 mL of M9 buffer and collect them in a 15 mL tube. Centrifuge the suspension at 800 x *g* for 2 min. Meanwhile, start preparing the bleaching solution.
    7. Take out the centrifuged tube and remove the M9 buffer with a 10 mL pipet. Add 10 mL of the bleaching solution and mix on the vortex until the adult nematodes are lysed. This takes usually approx. 5–7 min, but should not take longer than 15 min or the eggs will not develop fully later on in the experiment.
    8. Centrifuge the suspension at 800 x *g* for 2 min. Wash 3 times with 10 mL M9 buffer to clear the eggs from bleaching solution.
    9. Now add 150 µL of the egg suspension on OP50 plates. A density of 200–300 eggs should be reached on each plate. Wait until nematodes reached the adult stage.
    NOTE: The M9 buffer (pH 7.0) used for the depicted experiments consists of the following substances: 22 mM $KH_2PO_4$, 42 mM $Na_2HPO_4$, and 86 mM NaCl. After autoclaving the mixture, add 1 mM MgSO4. The bleaching solution contains 0.5 M NaOH, 2.3 mM NaOCl solved in distilled water.
    10. Prepare sterile M9 buffer, sterile pipette tips with a cut tip to wash off the worms, and 15 mL tubes to collect them in.
    11. Apply approximately 1 mL of M9 buffer on the plates, gently distribute the buffer by swinging and gently pipet the nematodes off the plate.
    12. Now, collect the nematodes in the tube. Apply 150 µL of suspension directly on the plates (seeded with OP50) with the cut pipette tips, and evaluate microscopically the density of the nematodes (recommended: 50–100 nematodes per 60 mm plate).
    13. If they are too dense, dilute the suspension with M9 buffer and evaluate it under the microscope until the desired density is reached. If the yield is too little, let the worms settle down or centrifuge the suspension 10 s at 800 x *g* to remove fluid.
    NOTE: An example of a sufficient density is given in **Figure 1A** (macroscopic) and **1B** (microscopic) at 20X augmentation. Proceed empirically as described.
    14. Keep in mind that the worms settle down quickly. To ensure an equal distribution between the plates, invert the tube after every stack of plates (4–5 plates).
    NOTE: The cut pipette tip, as well as the gentle swinging and washing of the plates, reduces shear stress.

## *2.* Transfer of Large Amounts of *Caenorhabditis elegans*

1. Wash off the nematodes with approximately 500 µL of M9 buffer, depending on the age and dryness of the plates. Using the same buffer, repeat detaching the nematodes until most of the animals are collected in suspension.
2. Slowly take up the nematodes with a cut pipette tip and transfer them on a plate prepared with OP50. Let the plate dry.
NOTE: Be careful not to apply much more than the recommended volume. Especially in long-term experiments, the plates might not tolerate it and the agar can break, allowing the nematodes to crawl into the cracks.

## 3. Harvest of a Large *Caenorhabditis elegans* Sample

1. Depending on the selected method, start experiments with a sufficient amount of *C. elegans*. For LC-MS/MS analyses, prepare 20 plates (60 mm x 15 mm) per group with approximately 100 nematodes per plate to ensure a yield of at least 200 µg of protein per sample.
NOTE: This part of the protocol does not require working under sterile conditions anymore, as the nematodes will be collected and frozen.
2. On the day of the sample collection, prepare liquid nitrogen to snap-freeze the collected samples. Keep non-sterile M9 buffer on ice to slow down the nematode's metabolism.
3. Wash off the *C. elegans* from the plates by applying 1 mL of M9 buffer and gently swirling the plates. This avoids collecting deceased nematodes and most eggs, if there are any present, because they stick to the bacteria and agar.

4. Take up the volume and transfer it to a 15 mL tube.
5. After the collection of the complete sample, wait for the nematodes to settle or centrifuge the tube briefly at 800 x *g* and remove most of the M9 buffer. Wash the sample 3x with approximately 10 mL of M9 (10x the sample volume) to remove any bacteria.
   NOTE: To ensure that all deceased nematodes are removed, sucrose floatation is another option. However, this was not suitable for the displayed experiments[15]. Sucrose is hydrolyzed to glucose and fructose. In the depicted experiments, the role of glucose was assessed to promote biochemical changes found in chronic diabetes. Thus, sucrose floatation could potentially confound the results.
6. Prepare one 1.5 mL tube with 1 mL of M9 buffer and one empty 1.5 mL tube for every sample. Transfer the pellet with a glass pipet from the 15 mL tube to the empty 1.5 mL tube. This step is crucial to ensure a quantitative transfer.
   NOTE: Unlike during previous steps, here the nematodes are more concentrated. Because of a possible adherence to plastic pipet tips, loss of a large part of the sample is expected. Therefore, use glass pipettes instead.
7. After the transfer, use the glass pipette to take up 1 mL of M9 buffer from the second 1.5 mL tube to rinse the nematodes from the pipette wall. Also, wash the remaining nematodes from the 15 mL tube and transfer them to the sample tube.
8. Centrifuge the sample tube at 19,000 x *g* for 1 min and then remove as much supernatant as possible.
9. Seal the tube with Parafilm and immediately snap-freeze it in liquid nitrogen. Store the tube at -80 °C until the lysate preparation.

## 4. Protein Isolation for Mass Spectrometry

NOTE: The protein isolation described here can also be used for other assays (*e.g.*, western blot).

1. To the frozen sample, add the same volumes of 0.5 mm zirconium oxide beads and at least 2x the volume of lysate buffer containing a proteinase inhibitor cocktail.
   NOTE: The depicted nematodes-to-protein correlation analyses were performed using 100 µL of buffer. LC-MS/MS samples were lysed with 200 µL of buffer.
2. Start the homogenizer for 1 min on speed 9. Ensure that the samples are completely lysed. Repeat this step if they are not fully lysed.
3. Centrifuge the samples for 30 min at 4 °C at 19,000 x *g*. Transfer the supernatant to a new tube on ice and store it at -80 °C until further measurements are going to be taken.
   NOTE: The non-denaturing buffer used for the depicted experiments consists of the following substances: 20 mM Tris HCl (pH 8), 137 mM NaCl, 1% Triton-X, and 2 mM EDTA.
   NOTE: Having followed the steps of this protocol and having applied the suggested scale, the yield of a protein pellet from approximately 1,000 nematodes should be approximately 500 µg. For more comparisons, consult **Figure 2** and the **Representative Results**.

## 5. Sample Preparation for Liquid Chromatography-tandem Mass Spectrometry Measurements

NOTE: Depending on the parameter of interest, the sample preparation will differ. This protocol focuses on methylglyoxal and AGE determination.

1. **Measure intracellular methylglyoxal by derivatization with 1,2-diaminobenzene (DB) as previously described[9].**
   1. Homogenize the samples with 20 µL of ice-cold 20% (wt/vol) trichloroacetic acid in 0.9% (wt/vol) sodium chloride and 80 µL of water in a 1.5 mL tube.
   2. Spike the samples of 5 µL with the internal standard ($^{13}C_3$-MG; 400 nM), mix them by vortexing, and then centrifuge them at 21,000 x *g* for 5 min at 4 °C.
   3. Transfer 35 µL of the supernatant to the HPLC sample vial containing a 200 µL glass insert.
   4. Add 5 µL of 3% sodium azide (wt/vol) to each sample followed by 10 µL of 0.5 mM DB in 200 mM HCl containing 0.5 mM diethylenetriaminepentaacetic acid (DETAPAC) in water.
   5. Incubate the samples for 4 h at room temperature, protected from light.
   6. Analyze the samples by LC-MS/MS, as previously described[9].
      NOTE: For the measurement of AGEs, isolate the proteins as described in section 4 of the protocol.

2. **Measure the protein concentration of the (thawed) lysates using a Bradford assay[10].**
   1. Prepare aliquots of 30 µL for a standard curve spiked with bovine serum albumin (BSA) solved in phosphate-buffered saline (PBS) of the following concentration: 0; 0.1; 0.2; 0.3; 0.4; 0.6; 0.8; and 1.0 mg/dL.
      NOTE: If the sample was prepared at the suggested size (see step 4.1 for the preparation of lysate with 200 µL of buffer), the estimated concentration should be in the range of approximately 2–7 mg/mL. In this case, dilute the samples 1:10 with PBS prior to the measurement.
      NOTE: For the first experiments using this method, it is recommended to measure the samples in different dilutions (1, 1:10, and 1:100). As there is no exact determination of the number of nematodes, the yield can differ between individual researchers.
   2. Use a transparent 96-well plate (polystyrene) and pipette 10 µL of each standard concentration and of the samples in the chosen dilutions in duplicates in the wells.
   3. Dilute the protein assay dye reagent concentrate 1:5 with distilled water (*aq. dest.*) and add 200 µL of the dilution to every sample on the plate. Incubate the plate for 10 min at room temperature.
   4. Measure the absorbance within 30 min at 595 nm with a plate reader. The samples can be interpolated from the calculated standard curve. More details of the method have been described extensively in the literature[10].

3. **Measure the intracellular AGEs as previously described[11,12].**
   1. Wash the total protein extracts (approximately 200 µg) 3x with water by ultracentrifugation in 10 kDa centrifugal filter units.
   2. Add 10 µL of 100 mM HCl, 10 µL of pepsin (2 mg/mL in 20 mM HCl), and 10 µL of thymol (2 mg/mL in 20 mM HCl) to the recovered protein (approximately 100 µL) and incubate the samples at 37 °C for 24 h.
   3. Neutralize and buffer the samples at pH 7.4 by adding 12.5 µL of 0.5 M potassium phosphate buffer (pH 7.4) and 5 µL of 260 mM KOH.

4. Add 10 µL of Pronase E [2 mg/mL in 10 mM potassium phosphate buffer (pH 7.4)] and 10 µL of a penicillin-streptomycin solution, and incubate the samples at 37 °C for 24 h.
5. Add 10 µL of aminopeptidase [2 mg/mL in 10 mM potassium phosphate buffer (pH 7.4)] and 10 µL of prolidase [2 mg/mL in 10 mM potassium phosphate buffer (pH 7.4)], and incubate the samples at 37 °C for 48 h.
6. Analyze the resulting hydrolysate by LC-MS/MS, as previously described[12].

## Representative Results

Here examples of creating a large-scale *C. elegans* culture for applications in diabetes research are presented. It can be of interest to relate the parameters to a single animal, rather than to normalize it to the total protein concentration. In an assay requiring a small number of nematodes, this can be easily accomplished by counting the nematodes. For a large-scale *C. elegans* culture involving hundreds of nematodes per experimental group, this approach is inconvenient. In **Figure 2**, the correlation between the amount of *C. elegans* and the protein content is shown. As demonstrated here, reproducible quantitative protein isolation can be achieved using this protocol.

The Amadori adduct fructosyl-lysine is one of several AGE precursors formed during diabetes in experimental animal models[13]. **Figure 3** displays LC-MS/MS measurements after a glucose treatment in 12-day-old nematodes and age-matched untreated controls from three independent experiments. The nematodes were homogenized with an addition of 200 µL of lysate buffer. An increased formation of fructosyl-lysine after the high-glucose treatment is presented.

Reactive metabolites such as methylglyoxal are fluctuant and modify proteins quickly[14]. Therefore, the question remains whether those reactive metabolites actually accumulate in the organism or are modified before reaching their target. **Figure 4** confirms the accumulation of methylglyoxal in *C. elegans* after a treatment with the substance.

Oxidative stress is increased during hyperglycemia[6], as well as during shear stress. **Figure 5** confirms no difference in the formation of oxidative stress between the glucose-treated group, transferred as in section 2 of the protocol, and the non-transferred group. Comparison of both glucose-treated groups to the untreated control group shows a significant increase in oxidative stress induced by glucose. In contrast, no significant difference between the two glucose-treated groups was observed. These results illustrate that handling nematodes using this protocol does not significantly induce the formation of oxidative stress, which could serve as a confounder in investigations of aging or metabolism.Moreover, findings in the current literature were reproduced using this protocol.

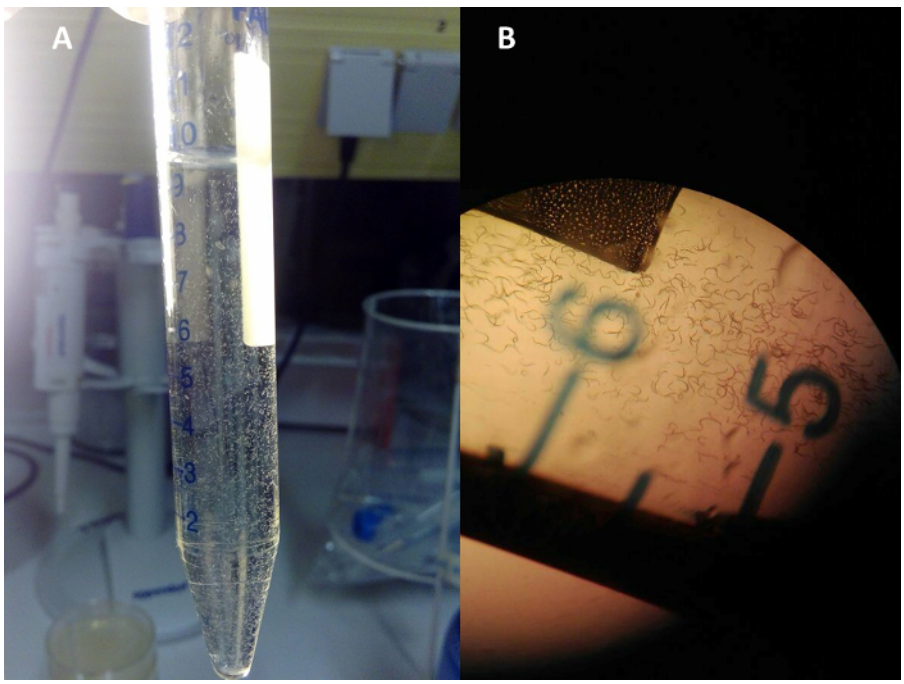


**Figure 1: Adequate density of nematodes for a further distribution on plates.** (**A**) This panel shows a macroscopic view. (**B**) This panel shows a microscopic view, at 20X augmentation. Please click here to view a larger version of this figure.

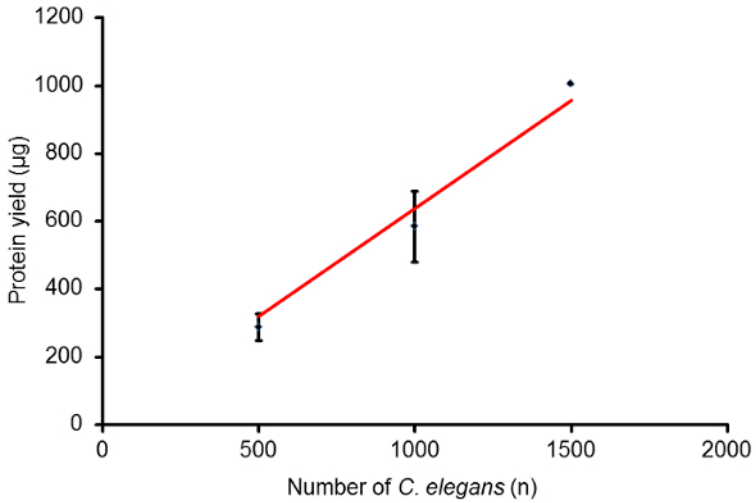


**Figure 2: Protein content of wild-type *C. elegans* N2.** This panel shows the correlation of the number of nematodes to the protein yield of the lysate, prepared as described in protocol section 5 in 12-day-old nematodes(*n* = 3 lysate of 500 nematodes; *n* = 3 lysate of 1,000 nematodes; and *n* = 1 lysate of 1,500 nematodes). The regression line is marked in red ($r^2$ = 0.976, *y* = 0.64). The total protein concentration was measured using the Bradford method. The data are expressed as the mean ± standard deviation (SD). Please click here to view a larger version of this figure.

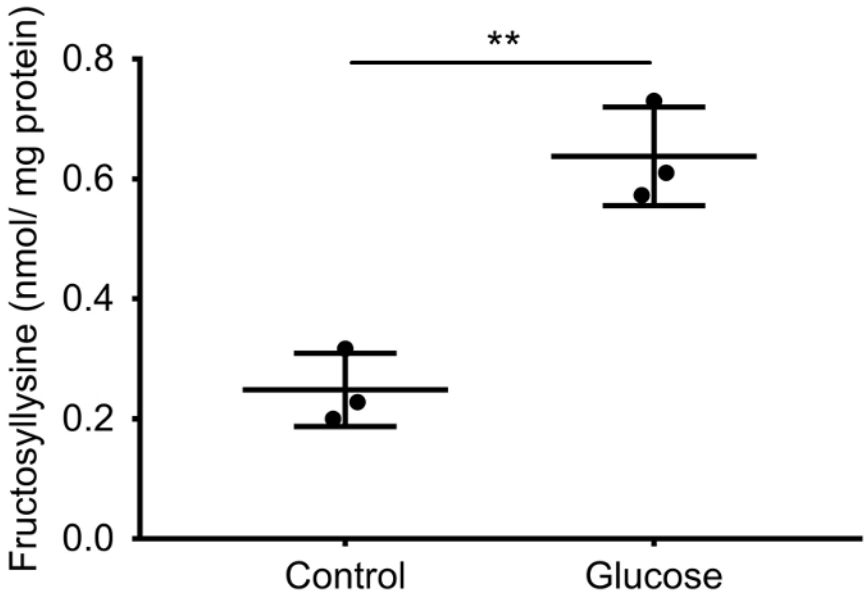


**Figure 3: Increased formation of fructosyl-lysine after a glucose treatment of *C. elegans* N2.** The concentrations of fructosyl-lysine after a treatment with glucose in 12-day-old nematodes and untreated controls were determined by LC-MS/MS and normalized to a protein concentration determined by the Bradford method. The data are expressed as the mean ± SD. The *p*-values were determined by Student's *t*-test, $^{**}p < 0.01$. The results are from three independent experiments. Please click here to view a larger version of this figure.

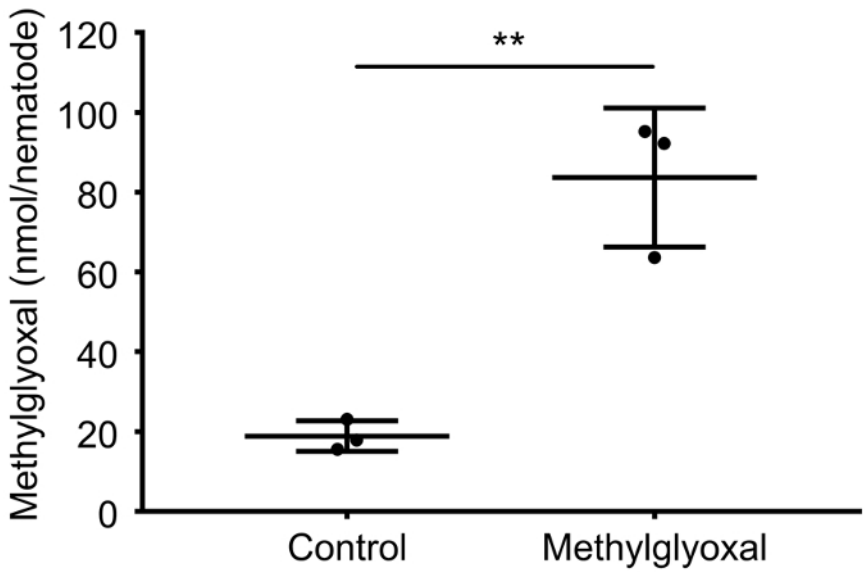


**Figure 4: Intracellular methylglyoxal concentrations after a methylglyoxal treatment of *C. elegans* N2.** Methylglyoxal concentrations were measured by LC-MS/MS in 12-day-old nematodes treated with methylglyoxal. The samples were collected less than 2 h after the last treatment. The data are expressed as the mean ± SD normalized to the number of nematodes. The *p*-values were determined by Student's *t*-test, $^{**}p < 0.01$. The results are from three independent experiments. Please click here to view a larger version of this figure.


**Figure 5: Oxidative stress measured in *C. elegans* CL2166 after a glucose treatment.** Transgenic *CL2166* (gst4::GFP)[8] containing a glutathione-S-transferase 4-promotor-driven GFP reporter were cultured on 400 µM FUdR plates containing glucose for 2 d. One group was transferred to fresh glucose plates on day 1 as described in section 2 of this protocol. Oxidative stress was measured as an increase in fluorescence [relative light units (RLU)] with a plate reader. The data are expressed as the mean ± SD. The *p*-values were determined by ANOVA, $^{*}p < 0.05$. The results are from three independent experiments. Please click here to view a larger version of this figure.

## Discussion

This protocol presents a reliable approach for the large-scale culturing of *C. elegans* to obtain quantitative results. Findings from the literature could be replicated as shown in the **Representative Results**. Even though this protocol for the collection of large-scale samples of *C. elegans* seems like a straight-forward method, there are certain pitfalls to take into account. Regarding the synchronization of the nematode population, this protocol describes an approach by bleaching the population with sodium hypochlorite and sodium hydroxide to destroy the nematodes and harvest the eggs solely. It has to be taken into account that this approach might not be suitable for all experiments. Depending on the ratio of the population size to the volume of the bleaching solution, the harming effect of the bleaching solution influences the development of the embryos[15]. Especially when studying developmental processes, this could be a crucial step. Concerning the handling of the nematodes, it is crucial to apply as little shear forces as possible throughout the protocol. If not handled with care, a large number of nematodes will perish. For that matter,

it is important not to exceed the spinning time and speed. When transferring the nematodes, a cut pipette tip to reduce the number of injured nematodes should be used. For the transfer of the nematodes, the recommended volume of buffer should not be exceeded, as the agar might crack when it becomes too damp, giving the nematodes the opportunity to dig into the plate. While collecting the samples, the M9 buffer must be kept ice-cold to slow down the nematodes' metabolism, especially when working with metabolites or substrates that *C. elegans* will degrade. It is important to wash the sample thoroughly to minimize any bacterial contamination from prior feeding. When transferring the pellet, remember that a great number of worms could stick to plastic pipette tips. The recommended glass pipette should be used, as even low-binding pipette tips could retain a large amount of the sample. As an alternative, the addition of 0.01% Triton-X100 to the M9 buffer was previously suggested[16]. To ensure that no deceased *C. elegans* or bacteria will influence the results, sucrose floatation after the collection of the nematodes can be performed[14]. This is usually done after liquid culturing to remove the medium. In this experiment, this cleansing was omitted, because sucrose is metabolized into glucose and fructose, thus potentially confounding our results.

Typically, a large-scale culture of *C. elegans* is performed using liquid media. Liquid culturing, however, is not suitable for all experimental settings, especially when using readouts which require exact numbers of nematodes. A modification of the Baerman apparatus was previously described to sort and purify nematodes derived from liquid culture[17]. This method greatly reduces bacterial contamination and filters only adult nematodes through a multiple component filter system. This elegant method is limited by the long filtering time (2 - 6 h) at room temperature, which could confound metabolic analyses. As for the filtering technique, nematodes are sorted by their moving activities. Nematodes have to be fit enough to crawl independently through the pores of filters from different materials. Thus, results could be distorted by excluding nematodes that are weakened by the experimental treatments.

Combining liquid and plate cultures in a single experimental design could also serve as a confounder in later analyses. *C. elegans* develops a thinner and longer phenotype in liquid culture, making it difficult to compare parameters normalized to mass, protein content, or body length and width[2]. Moreover, the study of reactive substances is challenging in liquid culture, since reactive substances will likely be modified or inactivated by media components before reaching the nematodes. Even though large-scale samples of *C. elegans* can be obtained with this protocol, plate culture itself is more labor-intensive than liquid culture. However, there are certain ways to facilitate the handling (*e.g.*, with the use of an agar-dispensing machine). Another option to promote large-scale culture efficiently is the use of Petri dishes with a larger diameter for the production of agar plates. This option might be constraint by the succeeding analyses. For microscopic assessment or plate handling in general, the amenities decrease with the diameter of the dish.

Multiple high-throughput approaches suitable for the assessment of different parameters, such as chemical or drug screening, have been developed and investigated[18]. Microfluidic devices allow researchers to study various parameters, as shown in a model of type-2 diabetes[19]. Lifespan, lipid metabolism, and oxidative stress responses can be simultaneously assessed on a single-animal level, revealing the advantages of high-content screening, which integrates phenotypic with biochemical information. The assessment of the reliability and reproducibility of the current literature has already shown a great refinement of these techniques, going beyond proof-of-concept studies[18]. The affordability, especially for smaller laboratories, still remains problematic, as the devices often have to be customized according to experimental needs, which can prove to be costly.

The quantification of AGEs in *C. elegans* is complicated by the impermeable cuticle of the nematode. A commonly used method is the detection of AGEs *via* immunofluorescent stainings[6]. Because of the cuticle, larger samples sizes are needed to decrease the high variance of the results. Imaging has to be taken into account as a time-consuming factor.

The protocol for whole-body lysate preparation described here makes intracellular AGEs and other components of *C. elegans* accessible for analyses. LC-MS/MS measurements of *C. elegans* samples have been performed before[14]. Adequate culturing conditions, however, to achieve a sufficient number of nematodes, have not been described in detail, yet.

The method described in this protocol is useful for readouts requiring a large-scale, homogenously grown population of *C. elegans,* or for the combination of multiple readouts per experiment. This is particularly convenient for studying complex multifactorial diseases such as diabetes and its complications. Alternative approaches, such as high-throughput and high-content screening using microfluidic systems, are technologically more advanced and are able to provide larger-sized data. Their main application can currently be seen in chemical and drug screening or genetic screening for mutants responding to the experimental treatments. This protocol helps researchers to easily and inexpensively upscale the size of their current or future projects without the need for an adjustment of the current experimental readouts and, thus, to minimize any time loss associated with the establishment of new methods.

## Disclosures

The authors have nothing to disclose.

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