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Article *in* Journal of Visualized Experiments · June 2022

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Small-scale Extraction of *Caenorhabditis elegans* Genomic DNA

Bhoomi Madhu[1], **Mohammed Farhan Lakdawala**[1], **Tina L. Gumienny**[1]

[1] Department of Biology, Texas Woman's University

Corresponding Author

Tina L. Gumienny
tgumienny@twu.edu

Citation

Madhu, B., Lakdawala, M.F., Gumienny, T.L. Small-scale Extraction of *Caenorhabditis elegans* Genomic DNA. *J. Vis. Exp.* (184), e63716, doi:10.3791/63716 (2022).

Date Published

June 7, 2022

DOI

10.3791/63716

URL

jove.com/video/63716

Abstract

Genomic DNA extraction from single or a few *Caenorhabditis elegans* has many downstream applications, including PCR for genotyping lines, cloning, and sequencing. The traditional proteinase K-based methods for genomic DNA extraction from *C. elegans* take several hours. Commercial extraction kits that effectively break open the *C. elegans* cuticle and extract genomic DNA are limited. An easy, faster (~15 min), and cost-efficient method of extracting *C. elegans* genomic DNA that works well for classroom and research applications is reported here. This DNA extraction method is optimized to use single or a few late-larval (L4) or adult nematodes as starting material for obtaining a reliable template to perform PCR. The results indicate that the DNA quality is suitable for amplifying gene targets of different sizes by PCR, permitting genotyping of single or a few animals even at dilutions to one-fiftieth of the genomic DNA from a single adult per reaction. The reported protocols can be reliably used to quickly produce DNA template from a single or a small sample of *C. elegans* for PCR-based applications.

Introduction

Here, two related protocols are presented for the lysis of *Caenorhabditis elegans* to make DNA accessible for PCR-based applications. PCR is a commonly used molecular technique used for many applications, including genotyping and amplifying DNA fragments for cloning and sequencing, among others. The small (1 mm), free-living roundworm *C. elegans* is a popular animal system for biological research. Obtaining suitable genomic DNA from a single animal or a few animals is sufficient to amplify the sequence by PCR. Late L4 larvae and adults contain only ~1,000 somatic cells (including some multi-nuclear, polyploid cells), germ cells, and (if the animal is a gravid hermaphrodite) offspring *in utero*[1]. However, these animals are protected by a cuticle that must be disrupted to extract the genomic DNA[2]. Standard methods to prepare nematode genomic DNA template for PCR involve multiple steps and take several hours. The animals are first frozen in worm lysis buffer containing proteinase K (−70 °C or below) for at least 15-45 min (longer is recommended by some protocols)[3,4,5,6]. This step cracks open the animals.

After freezing, the animals are incubated for 1 h at 60-65 °C for the proteinase K to work, then the enzyme is inactivated for 15-30 min at 95 °C. The proteinase K destroys the nucleases that degrade DNA. The inactivation of proteinase K before PCR is important to prevent the proteinase K from degrading the DNA polymerase. The two kit-based protocols described here are quick, reliable, and cost-effective methods to extract genomic DNA from either a single animal or a few nematodes for everyday research and teaching laboratory applications. The kit used was originally optimized by the manufacturer to extract DNA from animal tissue, saliva, and hair[7]. It uses a proprietary tissue preparation solution and extraction solution to lyse cells and make genomic DNA accessible. A proprietary neutralization solution then neutralizes the components that may inhibit PCR (e.g., salts, ions, and Mg^{2+}-binding molecules).

When genotyping, a single animal can be tested. When determining if a strain is homozygous, testing six or more offspring from a single animal gives high confidence that a line is homozygous or not (there is a 0.02% chance of randomly picking six homozygous mutant progeny from a heterozygous parent [$(1/4)^6 \times 100\% = 0.02\%$]). This method 1) is straightforward, with fewer steps than the proteinase K method, and 2) decreases the template preparation time to 15 min. The results in this work demonstrate that the developed protocol works robustly in extracting genomic DNA from single or a few worms, which can be reliably used for downstream applications that do not require highly purified DNA, including PCR.

Protocol

1. *C. elegans* maintenance

NOTE: N2 (wild type) and *blmp-1(tm548) C. elegans* strains were maintained on standard nematode growth media (NGM) plates at 20 °C.

1. Prepare NGM plates by dissolving 23.005 g of NGM powder in 973 mL of water in a 2 L flask. Cover the flask opening with aluminum foil (or autoclavable cap that allows venting) and secure the covering with autoclave tape. Autoclave to dissolve the powder and sterilize the contents with a sterilization cycle of at least 30 min.
2. Cool the medium to 60 °C in a water bath.
3. To the cooled medium, add 25 mL of sterile 1 M phosphate (KH_2PO_4) buffer, pH 6.0; 1 mL of 1 M calcium chloride solution; and 1 mL of 1 M magnesium sulfate solution.
4. Stir the medium on a magnetic stir plate to obtain a homogeneous mixture and to prevent uneven cooling and solidification (~5 min). Ensure that the stir bar spins fast enough to create a vortex but not so fast that bubbles are introduced (~250-300 rpm).
5. Pour ~25 mL of the medium into each 10 cm Petri plate (~40 plates in total). Dry the plates at room temperature overnight (typically 16-25 °C).
6. Grow a colony of *Escherichia coli* OP50 in 30-50 mL of LB broth overnight at 37 °C.
7. Seed each plate with 500 µL of *E. coli* OP50 culture and let them dry at room temperature before use (typically 16-25 °C)[8]. Store the plates at 4 °C for up to 3 weeks.

8. Transfer *C. elegans* to seeded plates using a wire pick or other method and let them grow to L4 or adult stage at the temperature required for the strain (typically 16-25 °C, 1-2 days if animals were plated starved as dauers, 3-4 days if animals were plated as embryos) [8].

2. Single-worm DNA extraction

NOTE: This method is useful for extracting DNA from a single worm (one worm in 1.8 µL of total volume). A master mix can be made if multiple worms will be lysed at one time.

1. Program a thermocycler for one cycle at 55 °C for 10 min followed by 95 °C for 3 min (lysis program).
2. Aliquot 0.8 µL of the extraction solution from the kit onto the inside wall of a 0.2 mL PCR tube on ice. Add 0.2 µL of the tissue preparation solution from the kit to the droplet of the extraction solution. Mix by pipetting.
3. Identify an L4 or adult animal under a dissecting microscope[9]. To identify L4 hermaphrodites, look for a characteristic vulva that is visible as a pale half-circle in the middle of the animal. Identify adult hermaphrodites by looking for the largest animals on the plate that may have oval embryos visible in their uterus.
4. Using a platinum wire pick, transfer the selected animal into the solution[10].
5. Centrifuge the tube briefly (2-3 s, ≤6,000 rpm/2,000 × *g*) at room temperature to collect the contents at the bottom of the tube.
6. Place the tube in the thermocycler and run the lysis program set at Step 2.1.
7. When the program is complete, briefly centrifuge the tube and place it on ice. Add 0.8 µL of the neutralization solution from the kit to the mixture. Mix by pipetting.
8. Centrifuge the tube briefly at room temperature (2-3 s, ≤6,000 rpm/2,000 × *g*).
9. Directly use the lysate for PCR or store it at 4 °C or −20 °C for future use.
   NOTE: Extracted DNA is stable at 4 °C or −20 °C for at least 6 months[7].

3. DNA extraction of a few individuals

NOTE: This method is useful for extracting DNA from a few worms. A master mix can be prepared if multiple strains will be lysed at one time.

1. Program the thermocycler for one cycle at 55 °C for 10 min followed by 95 °C for 3 min (lysis program).
2. Aliquot 2.0 µL of the extraction solution from the kit onto the inside wall of a 0.2 mL PCR tube on ice. Add 0.5 µL of the tissue preparation solution from the kit to the droplet of extraction solution. Mix by pipetting.
3. Identify L4 or adult animals under a dissecting microscope[9]. L4 hermaphrodites have a characteristic vulva that is visible as a pale half-circle in the middle of the animal. Adult hermaphrodites are the largest animals on the plate and may have oval embryos visible in their uterus.
4. Using a platinum wire pick, transfer a few animals into the solution: 9 animals yield 1 animal equivalent of genomic DNA per 0.5 µL of lysate, and 16 animals yield ~1 animal equivalent of genomic DNA in 0.25 µL (3.5 nematodes/µL)[10].
   NOTE: It is difficult to transfer greater than 16 animals into this volume.
5. Centrifuge the tube briefly at room temperature (2-3 s, ≤6,000 rpm/2,000 × *g*).

6. Place the tube in the thermocycler and run the lysis program set at Step 3.1.
7. When the program is complete, briefly centrifuge the tube and place it on ice. Add 2 µL of the neutralization solution from the kit to the mixture. Mix by pipetting.
8. Centrifuge the tube briefly at room temperature (2-3 s, ≤6,000 rpm/2,000 × *g*).
9. Directly use the lysis for PCR or store it at 4 °C or −20 °C for future use.
   NOTE: Extracted DNA is stable at 4 °C or −20 °C for at least 6 months[7].

4. PCR reaction

NOTE: One downstream application of this worm lysis technique, detecting a deletion mutation using a fast polymerase, is described. The effectiveness of the two worm lysis protocols in producing genomic template DNA for successful PCR at dilutions to 1/50th of a worm per reaction is also demonstrated.

1. Extract DNA from a single worm and 16 worms using wild-type N2 and mutant strains, as described above in Step 2 and Step 3.
2. Prepare 2-, 10-, 20-, and 50-fold dilutions of single-worm DNA from wild-type N2 animals.
3. Set up PCR reactions following the manufacturer's protocol using primers specific to the sequence of interest and hot-start PCR master mix (**Table 1** and **Table 2**). Use 1 µL of undiluted DNA or 2 µL of diluted DNA as the template in each reaction.
4. Confirm the PCR products by gel electrophoresis and imaging[11].

Representative Results

Genomic DNA from a single or a few wild-type adults was extracted using the commercial kit or traditional lysis protocol to compare the efficacy of these two methods. These lysates were then used as templates for PCR to amplify either a larger target of ~2,100 bp (encoding *blmp-1*) or a smaller target of ~500 bp (encoding a part of *sma-10*). Both methods successfully yielded appropriate PCR products (**Figure 1A**).

Next, the ability of kit-extracted genomic DNA to serve as template for a variety of DNA polymerases was demonstrated. Extracted genomic DNA was used as template for amplifying a 0.5 kb product using four polymerases that possess different capabilities (including speed, fidelity, and product size). Products of expected sizes were generated by these polymerases using template from a single-adult lysis or a nine-adult lysis (**Figure 1B**). The template sequence and fidelity of the PCR polymerases to correctly amplify the template sequence can be confirmed by sequencing (**Supplemental Figure S1**). This result demonstrates that the genomic DNA extracted from the kit protocols provides a suitable template for a range of polymerases.

PCR efficacy was tested using different concentrations of the genomic DNA extracted from a single animal using the commercial kit. DNA was diluted by 2-, 10-, 20-, or 50-fold, and the equivalent to about one-half, one-tenth, one-twentieth, and one-fiftieth of a worm per reaction was used for PCRs. These DNA template concentrations supported the amplification of either a larger target of ~2.1 kb or a smaller target of ~0.5 kb (**Figure 1C**)[12]. While a DNA concentration-dependent yield of the 0.5 kb PCR product was observed, the 2.1 kb PCR product yield appeared equivalent in all reactions.

To demonstrate that these protocols produce genomic DNA from *C. elegans* that is suitable for PCR genotyping, genomic DNA was extracted from single and 16 wild-type and *blmp-1* loss-of-function mutants (*blmp-1(tm548)*). The *blmp-1* gene encodes a transcription factor with important roles in distal tip cell migration, body size, and development[12,13,14,15,16]. The *blmp-1* mutant has an 810 bp deletion that removes parts of exon 3 and intron 3[15]. Primers were designed that result in a 2,134 bp product when the wild-type DNA template is used and a 1,324 bp product if *blmp-1(-)* DNA is used. PCR products of the appropriate sizes were detected using 1 µL of either single-worm (about 0.5 worms per reaction) or 16-worm lysis from L4 staged animals (3.5 worms per reaction) (**Figure 1D**). This result shows that kit-extracted genomic DNA using either protocol provides equally effective templates for PCR to genotype lines.

These results show that *C. elegans* genomic DNA preparations using a commercial tissue DNA extraction kit from single and multiple animals can be reliably used as templates for PCR.

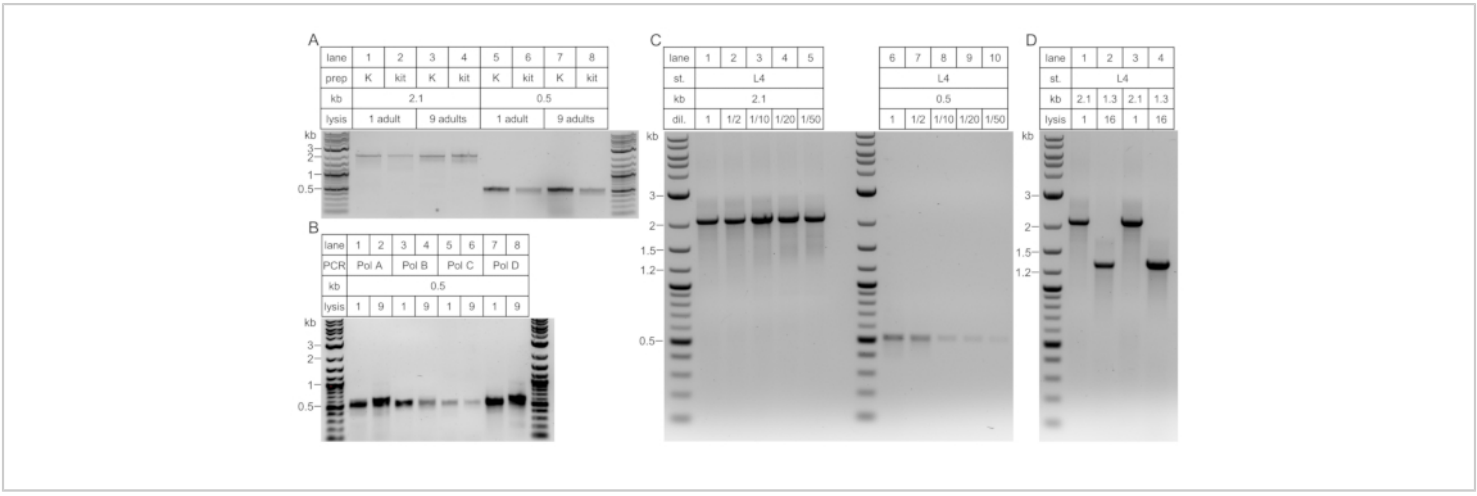

Figure 1: DNA preparations using a commercial kit from single nematodes and multiple nematodes allow robust amplification by PCR. PCR products were loaded on 1% agarose gel in 1x TAE buffer (5 µL (**A,B**) or 10 µL (**C,D**) and run at 90-100 V for 30 min to 2 h. A 2-log DNA ladder was used for all gels. (**A**) Comparison of DNA lysis by kit to traditional proteinase K methods to create template using two primer sets. Wild-type adults were lysed, and the equivalent of one animal was used as template for all reactions. Lanes 1-4, 2.1 kb product. Lane 1, PCR from single-worm lysis by proteinase K. Lane 2, PCR from single-worm lysis by the kit method. Lane 3, PCR from nine-worm worm lysis by proteinase K. Lane 4, PCR from nine-worm worm lysis by the kit method. Lanes 5-8, 0.5 kb product. Lane 5, PCR from single-worm lysis by proteinase K. Lane 6, PCR from single-worm lysis by the kit method. Lane 7, PCR from nine-worm worm lysis by proteinase K. Lane 8, PCR from nine-worm worm lysis by the kit method. (**B**) The kit method for DNA lysis provides a suitable template for PCR by multiple polymerases. Wild-type adults were lysed using the kit method, and the equivalent of one-half of an animal was used as PCR template for a 0.5 kb product. See the **Table of Materials** for polymerase details. Lane 1, PCR from a single-worm lysis with a high-speed polymerase. Lane 2, PCR from a nine-worm lysis with a high-speed polymerase. Lane 3, PCR from a single-worm lysis with a *Taq* polymerase. Lane 4, PCR from a nine-worm lysis with a *Taq* polymerase. Lane 5, PCR from a single-worm lysis with a high-fidelity polymerase. Lane 6, PCR from a nine-worm lysis with a high-fidelity polymerase. Lane 7, PCR from a single-worm lysis with a high-speed, high-fidelity polymerase. Lane 8, PCR from a nine-worm lysis with a high-speed, high-fidelity polymerase. (**C**) Dilutions of one wild-type L4 worm lysis provide template for PCR. Lanes 1-5, 2.1 kb product. Lanes 6-10, 0.5 kb target. Lane 1 and lane 6, undiluted DNA. Lane 2 and lane 7, 2-fold diluted DNA. Lane 3 and lane 8, 10-fold diluted DNA. Lane 4 and lane 9, 20-fold diluted DNA. Lane 5 and lane 10, 50-fold diluted DNA. (**D**) Genotyping the *blmp-1* locus by PCR using 1 µL of single- and 16-worm DNA preparations as template. Lane 1, PCR from wild-type single-worm lysis. Lane 2, PCR from *blmp-1(-)* single-worm lysis. Lane 3, PCR from wild-type 16-worm lysis. Lane 4, PCR from *blmp-1(-)* 16-worm lysis. Abbreviations: prep = method of preparation; kb = kilobase; st. = nematode stage; dil. = dilution of DNA. Please click here to view a larger version of this figure.

Target	Primer name	Sequence
blmp-1	forward	GCGTCAGGTAAGTTGTGATA
	reverse	CAGTTATTGCGGCTGTTGTT
sma-10	forward	AATGTGTGGCAAGGAATCG
	reverse	TGTCTGTCCAACGAGAAGTG
sequencing	1	CACATCCCGGACGCCTTAAT
	2	CTAATTGTTTTCTACCTGGC

Table 1: List of primers.

A.	Pol A, Pol B, Pol D	Pol E
PCR Master Mix	12.5 µL	12.5 µL
Forward primer	0.2 µM (final concentration)	0.5 µM (final concentration)
Reverse primer	0.2 µM (final concentration)	0.5 µM (final concentration)
template DNA	variable	1 µL
Nuclease-free water	to 25 µL	to 25 µL

B.	Pol C
PCR 5x buffer	2.5 µL
10 mM dNTPs	2.0 µL
Forward primer	0.2 µM (final concentration)
Reverse primer	0.2 µM (final concentration)
template DNA	variable
polymerase	0.5 µL
Nuclease-free water	to 25 µL

C. PCR program used for **Figure 1B**		
temperature	time	cycles
98 °C	2 min	1
98 °C	1 min	30
55 °C	1 min	
72 °C	1 min	
72 °C	1 min	1
4 °C	hold	

D. PCR program used for **Supplemental Figure S1**		
temperature	time	cycles
98 °C	30 s	1
98 °C	10 s	30

57 °C	20 s	
72 °C	50 s	
72 °C	2 min	1
4 °C	hold	

Table 2: PCR reaction components.

Supplemental Figure S1: Sequencing for confirmation of the quality of genomic DNA prepared with a commercial kit. The results from two sequencing reactions completely match the expected sequence of over 1,600 nucleotides of DNA amplified by a high-speed, high-fidelity polymerase (Pol E) from a 16-worm lysis (**Table 1**, **Table 2A**, and **Table 2D**). Sequencing read ends were trimmed to remove low-quality base reads. Alignment was performed using the EMBL-EBI multiple sequence alignment tool MUSCLE (MUltiple Sequence Comparison by Log-Expectation)[17]. Please click here to download this File.

Discussion

Determining the genotypes of *C. elegans* is an important step while performing genetic crosses to create new *C. elegans* strains. Genomic DNA extraction using a single or few *C. elegans* is a crucial step in genotyping *C. elegans*. This protocol describes genomic DNA extraction from *C. elegans* using a commercial kit. This method is fast and works robustly. The genomic DNA extracted using this method can be used for downstream applications, including genotyping, sequencing, and cloning. The described DNA extraction method has been optimized for genotyping *C. elegans* genetic crosses using single and a few L4 or adult animals as starting material for the DNA template. The equivalent of one-fiftieth of an animal (**Figure 1C**) to 3.5 animals (**Figure 1D**) provided a suitable template for amplifying target DNA sequences by PCR. This protocol (with dilution) yields enough template (at 2 µL/reaction) for a maximum of 45 reactions from a single worm and for 100 reactions from 16 animals. Even using one worm per reaction, these protocols provide a cost-efficient way to extract DNA from *C. elegans*. Given the simplicity, robustness, and quickness of the protocol, it is well suited for both research and classroom applications.

As the protocol involves pipetting small volumes of reagents, the preparation of a master mix for multiple reactions, good pipetting technique, and using well-calibrated pipettes is recommended to ensure consistency. Using 0.5-1 µL of undiluted DNA template from either single- or 9-16-animal lyses usually works for a 25 µL PCR reaction using a hot-start PCR master mix, but optimization of the template concentration may be required. The reagents must be kept on ice for the duration of the experiment. To avoid repeated freeze-thawing of the DNA extraction solutions, which may reduce enzyme performance, it is recommended that the reagents be aliquoted and stored at −20 °C.

For downstream applications requiring PCR, the use of a hot-start, high-speed PCR master mix further reduces the total time compared to the PCR reaction mix provided in the commercial tissue kit. High-speed polymerases can amplify products of less than 1 kb in 5-10 s (see the **Table of Materials** and **Table 2** for polymerase descriptions), while the kit's PCR reaction mix uses a *Taq* DNA polymerase with an

amplification rate of ~1 kb/min[7]. Products from some PCRs can be directly loaded into an agarose gel for electrophoresis, further reducing the steps and time. This reaction buffer is also compatible with restriction enzyme digestion. High-fidelity DNA polymerases also work robustly with DNA extracted using this protocol (**Figure 1B**). While the polymerases used consistently worked using kit-extracted template, the use of other polymerases may provide better results depending on the template, primer set properties, product size, and fidelity required. If problems are encountered following PCR, such as no target band amplified or additional bands amplified, further optimization of the primer working conditions or DNA template concentration may be required. It is strongly recommended that appropriate controls, including wild-type DNA template and the use of primers that have been proven to work, should be used.

This method will not be suitable for applications that require highly purified or a high yield of DNA template. While the number of animals prepared and the reaction volume may be scaled up, the DNA prepared using this method is not separated from organismal debris and may not be pure enough for highly sensitive applications such as whole-genome sequencing.

The major advantages of this method in comparison to the existing traditional genomic DNA extraction methods include the shorter time and simpler, easier steps. In the future, this method could be scaled up, adapted to extract *C. elegans* genomic DNA for other downstream applications, and used to extract DNA from other nematode species.

Disclosures

The authors have no conflicts of interest to disclose.

Acknowledgments

The N2 strain and *E. coli* OP50 bacteria were obtained from the *Caenorhabditis* Genetics Center (CGC), which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). The *blmp-1(tm548)* strain was obtained from the National Bioresource Project, Japan. The authors thank WormBase. This work was supported by NIH R01GM097591 to T.L.G., internal funding by Texas Woman's University to T.L.G, and TWU's Center for Student Research to M.F.L.

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