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Single worm lysis

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1 Works for me dx.doi.org/10.17504/protocols.io.587g9zn

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ABSTRACT

Exraction of genomic DNA (gDNA) from single adult *C. elegans* suitable for downstream molecular biology applications such as PCR.

MATERIALS

NAME	CATALOG #	VENDOR
Proteinase K		

MATERIALS TEXT

Proteinase K from *Tritirachium album*

lyophilized powder, ≥30 units/mg protein

Made up to 20mg/ml in Tris-HCl

1 Prepare 1mL 1X PCR buffer with 0.4mg/ml Proteinase K:

- **855 µl water**
- **95 µl 10X PCR buffer (pH8.3)**
- **50 µl 20mg/ml Proteinase K solution**
- **1000 µl Total volume**

nb. volumes can be scaled as required

10X PCR buffer (pH8.3) preparation:

- **18.64 g Potassium chloride (KCl)**
- **3.7 g Trizma base**
- **3.07 g Tris-HCl**
- **0.71 g Magnesium Chloride (MgCl2)**
- **500 ml water**

Autoclave

Cool (temperature affects pH)

pH meter and add hydrochloric acid to achieve the correct pH

2 Prepare worm lysis tubes with PCR buffer and allow **15 µl 1X PCR buffer + PK** per worm.

Pipette buffer into lid of PCR tube (up to 45ul can fit into tube lid)

3 Pick a single worm at a time into the liquid in the 200uL PCR tube. Make sure you can see the worm swimming in the buffer

4 Close the lids onto the tubes of the 200uL tube and spin in a microfuge so that the liquid and worms are at the bottom of the tube

5 Burst the cells open by freezing the tubes in the -80^{O}C freezer for 10 minutes

6 Transfer tubes to thermocyler and incubate on worm lysis program:
60^{O}C - 1hr 10 mins
95^{O}C - 15 mins

7 Store gDNA in -20^{O}C freezer until required. Use

2ul per 25uL PCR reaction.

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