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Single worm lysis

Ida Barlow¹

¹Imperial College London

Behavioural Genomics

Ida Barlow

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Protocol status: Working
 We use this protocol and it's working

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ABSTRACT

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

MATERIALS

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Proteinase K Contributed by users

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 μ L water

- 🧪 95 μ L 10X PCR buffer (pH8.3)
- 🧪 50 μ L 20mg/ml Proteinase K solution
- 🧪 1000 μ L Total volume

nb. volumes can be scaled as required

Note

10X PCR buffer (pH8.3) preparation:

- 🧪 18.64 g Potassium chloride (KCl)
- 🧪 3.70 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 μ L 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°C freezer for 10 minutes
- 6 Transfer tubes to thermocycler and incubate on worm lysis program:
60°C - 1 hr 10 mins
95°C - 15 mins
- 7 Store gDNA in -20°C freezer until required. Use
2ul per 25uL PCR reaction.